Toxicity Related to the Treatment of Pulmonary Tuberculosis

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UCL

MD(Res) Thesis

Author Declaration for Thesis

I, Conor Duncan Tweed confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Dr Conor Duncan Tweed Date

Thesis Abstract

Introduction

The incidence and nature of toxicity related to tuberculosis (TB) treatment, who is most commonly affected and what is the true impact on treatment is predominantly characterised through retrospective or observational studies; with varying definitions for toxicity and estimates of the incidence or patient groups at highest risk. REMoxTB was a randomised, controlled, phase III pulmonary TB clinical trial with stringent collection of efficacy and safety data.

Methods

A total of 639 patients received standard TB therapy as a control arm for the trial, with 655 patients and 636 patients allocated to the “isoniazid” and “ethambutol” arms. Related grade 3 and 4 adverse events were used to investigate the general toxicity observed during treatment and the liver biochemical tests collected were described in detail separately. Regression techniques investigated the association between patient demographics and toxicity. Lastly, the incidence of adverse events and the longitudinal pattern of clinical and laboratory data was described for HIV positive patients.

Results

Approximately 10% of patients experienced clinically significant toxicity attributed to their drug therapy. Older patients, female patients, those of Asian ethnicity, and HIV positive patients were at the greatest risk for toxicity. Significant drug toxicity most commonly occurred in the first two months of treatment. Liver dysfunction was the most frequent clinically significant toxicity. Patients who experienced one or more

episode of clinically significant toxicity were at higher risk of failing treatment. Those receiving the experimental moxifloxacin-containing arms experienced lower rates of clinically significant toxicity.

Conclusions

This work identifies patient groups who are at higher risk of toxicity, when this toxicity is likely to occur, and what form it most commonly takes: informing the allocation of often limited resources to appropriately monitor those patients who are at greatest risk during treatment. Additionally, by demonstrating a higher risk of failing treatment associated with drug toxicity, this work also provides further justification for changes at the policy level. Finally, the experimental arms could have a place in the management of patients at high risk of toxicity but this must be viewed in light of uncertainty surrounding the optimal duration of therapy.

Impact Statement

Standard TB therapy, while effective for the majority of patients, is acknowledged as potentially toxic and patients must know when to seek medical attention. The analyses presented here provide reassurance that many side effects from standard TB therapy are not life-threatening; however, there were several cases of extremely high liver enzyme elevations with associated symptoms of gastrointestinal upset and abdominal pain. It is critical that patients are made aware of the symptoms of hepatotoxicity and the importance of prompt medical intervention.

The information in this thesis will assist clinicians in making informed decisions regarding the monitoring of at-risk individuals. The identification of increased risk of toxicity among female patients, those with HIV infection, and Asian patients allows TB clinicians to target patients at greater risk of a complicated treatment course and take appropriate steps (for example, more frequent clinical reviews) to ensure an adequate package of care is in place.

The data presented here demonstrate that the majority of patients with liver enzyme elevations of greater than 3x upper limit of normal (ULN), but less than 5xULN, completed standard TB therapy and were not withdrawn from the trial. This would suggest that a practice of close monitoring but continuing treatment when liver enzyme levels are >3xULN but <5xULN would be reasonable in the absence of concerning features.

Episodes of clinically significant toxicity were associated with an increase in the odds of a patient failing to achieve a cure. Patients who fail to be cured of their TB can either remain infectious or return to an infectious state, are at a higher risk of developing

drug-resistant infections, and also are unlikely to remain fit to continue working. The first two risks are of public health concern as they will directly work against any efforts to curb or eradicate TB, and the third is a well-recognised economic issue associated with TB disease. This thesis would support the increased availability of liver function testing for all TB programs and regular testing to ensure significant elevations are detected early. The incidence of significant liver enzyme elevations in the intensive phase will help guide the timing for when these resources should be made available.

The experimental treatment arms had fewer patients reporting clinically significant adverse events compared to the standard TB therapy arm. The main implication of this finding would be the potential for the moxifloxacin-containing regimens to be adopted as treatment options for patients who are at elevated risk of experiencing adverse effects from treatment (for example, those with advanced liver disease, other significant pathologies, or an intolerance of isoniazid). However, any possible benefits of using these less toxic regimens must be carefully balanced against their failure to achieve formal non-inferiority compared to standard TB therapy, and the uncertain optimal duration of these experimental arms.

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Chapter One: Introduction

1 Historical Perspective on *Mycobacterium tuberculosis*

Pulmonary tuberculosis has claimed the lives of countless individuals since the genus *Mycobacterium* first originated over 150 million years ago (Hayman, 1984). The destructive lung disease caused by the infection has gone by many names throughout history (White Plague, phthisis, consumption) and in the 18th and 19th centuries it was crowned “Captain Among these Men of Death” across Europe and North America (Daniel, 2006). Celebrity has afforded no special privileges and tuberculosis has claimed the lives of many famous individuals, who in many cases continued to work under the burden of the infection that would ultimately kill them, including Emily Brontë, John Keats, Frédéric Chopin, and George Orwell.

On March 24 1882, Herman Heinrich Robert Koch stood at the podium in front of the Berlin Physiological Society and delivered his famous presentation *Die Aetiologie der Tuberculose* (Koch, 1932; Daniel, 2005). This was the first time that *Mycobacterium tuberculosis* had been identified as the infectious agent responsible for causing tuberculosis and the death of millions across the world. The discovery of the organism responsible did not lead directly to an effective cure, but sputum smear microscopy (followed by fluorescence microscopy) was then introduced as a test for the diagnosis of pulmonary tuberculosis (Hagemann, 1938; Steingart *et al.*, 2006).

The incidence of tuberculosis began to decline in the middle of the 19th century across Europe and North America, despite the lack of a pharmacologic cure for the disease (see Figure 1-1). Social conditions began to improve during this period, with the

introduction of basic hygiene measures such as sanitary disposal of human waste, and this had a significant impact on the transmission of all infectious disease including tuberculosis (McKeown and Record, 1962). There was increased access to safe and nutritious food sources from the beginning of the 19th century due to improvements in the ability to farm, transport and store food. An impaired nutritional status is linked to a reduction in the effectiveness of the immune system and an increased risk of active tuberculosis in endemic areas; this improvement in nutrition is believed to have been another factor in the reducing incidence of the disease (McKeown and Record, 1962; Wilson, 1990). Another factor that contributed to the reduction in the burden of TB disease in the UK was the improvement in the quality of city housing. An effort was made at the level of government to reduce overcrowding in public housing from the late 19th century, and this is credited with helping reduce the burden of disease by providing better ventilated and more hygienic living spaces for people from lower socioeconomic groups, among whom TB wasmost prevalent (McKeown and Record, 1962; Wilson, 1990). Finally, in the early 20th century it became routine practice to segregate patients suffering from active tuberculosis in sanatoria and this later intervention will likely have contributed further to the decline in the number of new cases by curbing the number of patients actively spreading infection (Vynnycky and Fine, 1997).

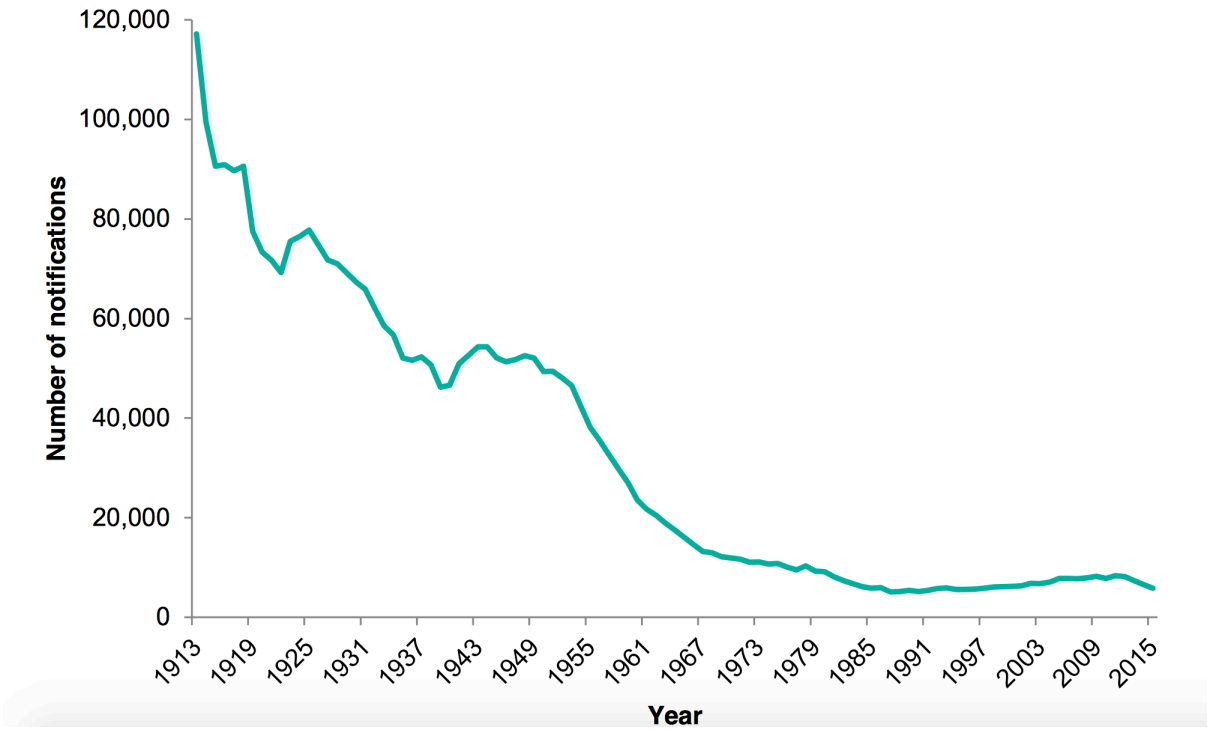

Figure 1-1. *Notification rate for tuberculosis affecting any site from 1913 to 2015 in England and Wales. Graph obtained from Public Health England Tuberculosis: annual notification and mortality data (1913 onwards) [https://www.gov.uk/government/publications/tuberculosis-tb-annual-notifications-1913-onwards]*

While the medical community was encouraged by the reduced number of tuberculosis cases seen across Europe and North America the fall began from a high incidence and pulmonary tuberculosis remained a major public health issue in the first half of the 20th century (Vynnycky and Fine, 1999). Additionally, while significant progress was being made in settings with adequate resources, progress was much slower in other parts of the world. By the middle of the 20th century it was becoming clear that tuberculosis incidence figures and outcomes were diverging at global economic boundaries: the tuberculosis epidemic continued largely unabated in low income settings where the available healthcare resources fell far short of what was required (Keshavjee and Farmer, 2012).

2 Tuberculosis Chemotherapy

The discovery of para-aminosalicylic acid (PAS) in 1943 and the isolation of streptomycin a year later brings the narrative into the current era of tuberculosis

chemotherapy (Medical Research Council, 1948; Marshall *et al.*, 1950). The next three decades would see the development of an effective but lengthy treatment regimen for pulmonary tuberculosis (Fox, Ellard and Mitchison, 1999). However, at the time of writing it is the most common cause of death from an infectious disease world-wide (WHO, 2017) and the End TB Strategy proposed by the World Health Organisation to curb the epidemic has continued to fall short of its stated goals (Floyd *et al.*, 2018).

The road that led to the current standard tuberculosis therapy began with what is considered to be the world's first randomised controlled trial in 1947 (Medical Research Council, 1948). The British Medical Research Council (BMRC) recruited 107 patients with confirmed pulmonary tuberculosis and randomly allocated them to receive either regular streptomycin injections or bedrest alone (see Figure 2-1). There were only 4 deaths in the group receiving streptomycin and 14 deaths in the control group, which represented an unprecedented success in the treatment of tuberculosis. However, the majority of the patients treated with streptomycin relapsed (Crofton and Mitchison, 1948) and by 1950 it was apparent that single-agent therapy led to the development of bacterial resistance, even in patients who were apparently cured at the time (Fox, Sutherland and Daniels, 1954). The next two decades were spent in a flurry of activity as more drugs with activity against tuberculosis were brought into widespread use (isoniazid, rifampicin, and pyrazinamide in particular), the first standardised 18 to 24 month regimen for treating tuberculosis was developed, management of the disease was brought out of hospital and into the community, and treatment was eventually shortened from 18 to 6 months (Fox, Ellard and Mitchison, 1999; Mitchison and Davies, 2012).

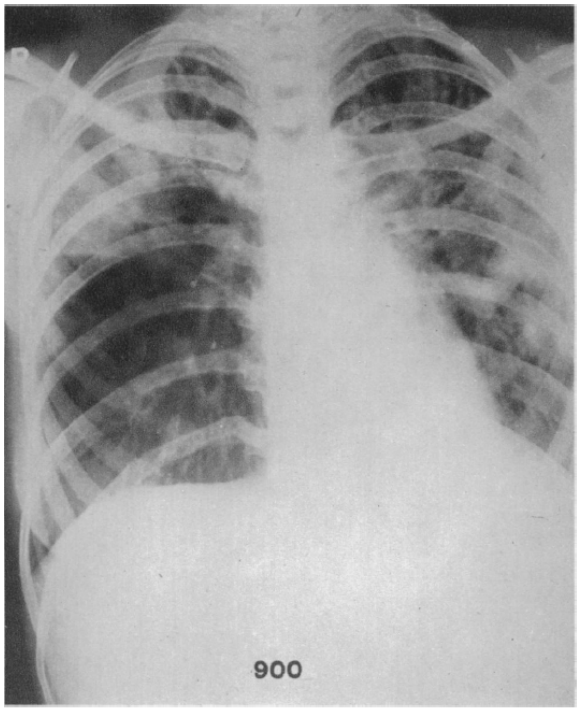

FIG. 3.—Case 90 (S). April 26, 1947.

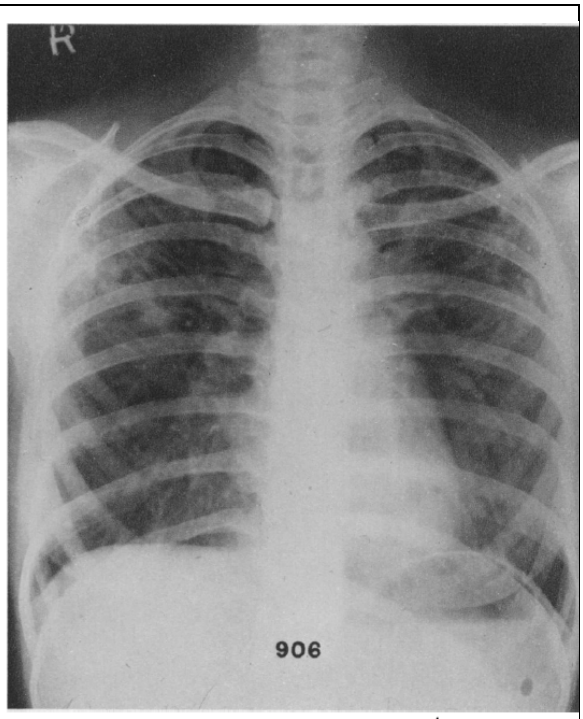

FIG. 4.—Case 90 (S). Nov. 5, 1947.

Figure 2-1. *Chest X-rays for the same patient prior to treatment and after treatment with streptomycin in the first British Medical Research Council trial to investigate the effects of treatment in 1948. [British Medical Research Council. Streptomycin Treatment of Pulmonary Tuberculosis. Br Med J 1948;30:769-782]*

Following the success in using a combination of rifampicin and pyrazinamide to shorten treatment down to 6 months, attempts were made to shorten treatment even further. A series of trials in Eastern and Central Africa, Hong Kong, Singapore, Madras, and Algeria demonstrated that reducing treatment to less than 6 months resulted in an unacceptable rise in the number of relapsed infections (Fox, Ellard and Mitchison, 1999). These trials also used different combinations of drugs over the course of the 6 months to identify the most effective therapy, and this was in part driven by a desire to reduce the cost of treatment but also to reduce the risk of drug-related toxicity. The sterilising activity of rifampicin was identified as persisting through the full 6 months of treatment and was therefore essential to treating tuberculosis with a “short course” of treatment (East African/British Medical Research Council, 1973). Conversely, pyrazinamide’s activity seemed to be confined to the first two months of therapy (East

African/British Medical Research Councils Study, 1981; East and Central African/British Medical Research Councils, 1986; Fox, Ellard and Mitchison, 1999).

The increasing availability and use of tuberculosis chemotherapy coincided with the ongoing decrease in the number of notified cases of tuberculosis from approximately 20,000 new cases in 1955 to approximately 5,000 cases in 2015 across England and Wales (Figure 1-1). Untreated tuberculosis carries an approximately 70% chance of death (Tiemersma *et al.*, 2011) and, while much can (and should) be made of the importance of social reform in the eradication of tuberculosis, active treatment has an impact at both the individual patient level and from the public health perspective. The earliest treatment regimens (although lengthy) did contribute to the fall in case fatality from between approximately 40-70% in untreated disease to less than 10% among treated patients in both resource-rich and resource poor settings (Hermans, Horsburgh and Wood, 2015) (Figure 2-2).

Current tuberculosis chemotherapy's public health impact results from over 90% of the mycobacteria dying within two weeks of starting treatment (Donald and Diacon, 2008) as this reduces the ability of the infection to spread through aerosol transmission from the host (Brooks, Lassiter and Young, 1973; Fennelly *et al.*, 2004). Although it should be noted that there is some challenge to a dogmatic approach to this time period, as some patients will become less infectious earlier and later than two weeks (Escombe *et al.*, 2007; Fennelly, 2007; Churchyard *et al.*, 2017). The World Health Organisation has endorsed the Directly Observed Treatment, Short-course (DOTS) approach to administering treatment as part of tuberculosis control strategies since the early 1990s (WHO 2011); however, there is evidence that suggests there is no significant difference between DOTS and self-administered treatment in terms of treatment outcomes (Karumbi & Garner 2015; Pasipanodya & Gumbo 2013).

Figure 2-2. *Line graphs illustrating the reported TB mortality (Graph A) and case fatality (Graph B) rates over time in London, New York and Cape Town from 1910 to 2012. Rates from 1913 to 1965 are for London County Council (current Inner London) and thereafter refer to data relating to the Greater London Area (Inner and Outer London). [Hermans et al. A Century of Tuberculosis Epidemiology in the Northern and Southern Hemisphere: The Differential Impact of Control Interventions.PLoS ONE 2015;10(8):e0135179]*

Standard tuberculosis therapy for drug-sensitive disease now consists of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) for two months followed by isoniazid and rifampicin for a further four months (2HRZE/4HR). This regimen has a relapse-free cure rate of over 90% in clinical trial conditions (Fox, Ellard and Mitchison, 1999) and it has been used in an essentially unchanged format for over 25 years.

From the early 1980s the prevailing belief was that all the necessary tools were available to end the epidemic, and it would not be long before tuberculosis would obligingly melt away.

3 Tuberculosis and the Human Immunodeficiency Virus

It is thought that sometime in the 1930s the Simian Immunodeficiency Virus (SIV) was transmitted from its ape hosts to bushmeat hunters in the Democratic Republic of Congo (Sharp and Hahn, 2011). This initial transmission would go on to become Human Immunodeficiency Virus 1 (HIV-1), and is the most widespread form of the virus found today (Maartens, Celum and Lewin, 2014). The infection is most commonly spread between humans through sexual contact, and over the first several decades of human-to-human transmission it appears to have been relatively contained (Hutchinson, 2001). However, genetic analysis of HIV suggests that the virus first arrived in the United States of America in the late 1960s and over the course of the 1970s a global pandemic silently unfolded (Robbins *et al.*, 2003).

The early 1980s saw a high prevalence of Kaposi's sarcoma and pneumonia detected among men who have sex with men in New York and California (Altman, 1981; Anon, 1981). In 1982, the Centre for Disease Control in Atlanta established the term "Acquired Immune Deficiency Syndrome" (AIDS) and an infectious aetiology was assumed despite no organisms having been identified (Merson *et al.*, 2008). Other groups at risk were identified, including injecting drug users and haemophiliacs (Altman, 1981; Anon, 1982; Merson *et al.*, 2008). By the end of 1982, AIDS had been detected on five continents and the scientific community became aware that the so-called "slim disease" seen on the African continent was, in fact, AIDS as well (Merson

et al., 2008). At the close of the decade, it was estimated that up to 10 million people could be infected with HIV across the globe and the World Health Organization launched its Global Program on AIDS to tackle the pandemic (Mann, 1987). Over the next 20 years, efforts toward controlling the HIV epidemic led to the discovery of the virus responsible, and the development of effective treatment that can suppress the infection and reduce transmission of the virus (Barré-Sinoussi, Ross and Delfraissy, 2013).

HIV is one of the main reasons behind our failure to control the global tuberculosis epidemic. The virus acts to suppress the host immune system, and therefore leaves the patient vulnerable to infection (Deeks *et al.*, 2015). This particularly applies to tuberculosis as HIV impairs the activity of several immune cells that are essential for the containment of tuberculosis in the lung parenchyma (Bell and Noursadeghi, 2017). Groups who are at higher risk of contracting HIV include men who have sex with men, sex workers, prisoners, and injecting drug users (Maartens, Celum and Lewin, 2014). The HIV epidemic was associated with an increase in the number of cases of both pulmonary and extra-pulmonary tuberculosis across Europe and North America in the 1980s and 1990s, particularly among these higher-risk groups, as at this time there was no effective treatment available to suppress HIV (Merson *et al.*, 2008).

In low- and middle-income countries efforts to control tuberculosis had met with limited success before the HIV pandemic, and now they were faced with an even greater challenge with the overlap of the two infections (Chaisson and Martinson, 2008). As a result of both social and political factors the highest prevalence of people living with HIV (PLHIV) can be found in Africa (UNAIDS, 2017) (reported as an estimated 25.6 million individuals in 2016, see Figure 3-1). In particular, the eastern and southern regions of the continent are home to a very large number of PLHIV (UNAIDS, 2017)

(estimated at 19.4 million individuals in 2016). This distribution of HIV is reflected in the global tuberculosis figures: the WHO Africa region contains the highest number of co-infected (TB-HIV) patients and out of approximately 500,000 incident tuberculosis cases in South Africa over 50% are also HIV-positive (WHO, 2017). It is estimated that 10% of the 10.4 million incident tuberculosis cases in 2016 were co-infected with HIV, and tuberculosis-related deaths accounted for approximately 340,000 of the 1 million deaths among all HIV-infected patients (UNAIDS, 2017; WHO, 2017). Despite a world-wide decrease in case fatality from HIV-associated tuberculosis (largely attributable to the roll-out of ART and earlier identification and treatment of tuberculosis) many PLHIV in low- and middle-income countries die from undiagnosed TB (Cohen *et al.*, 2010). While anti-retroviral therapy (ART) does lower the risk of developing tuberculosis in PLHIV, and there is an association with an overall reduction in the risk of treatment failure in retrospective and observational work (Manosuthi *et al.*, 2006; Sungkanuparph *et al.*, 2006; Zhou *et al.*, 2009), despite significant gains in the roll-out of ART globally there are still many areas where treatment coverage is below 50% of PLHIV (UNAIDS, 2017).

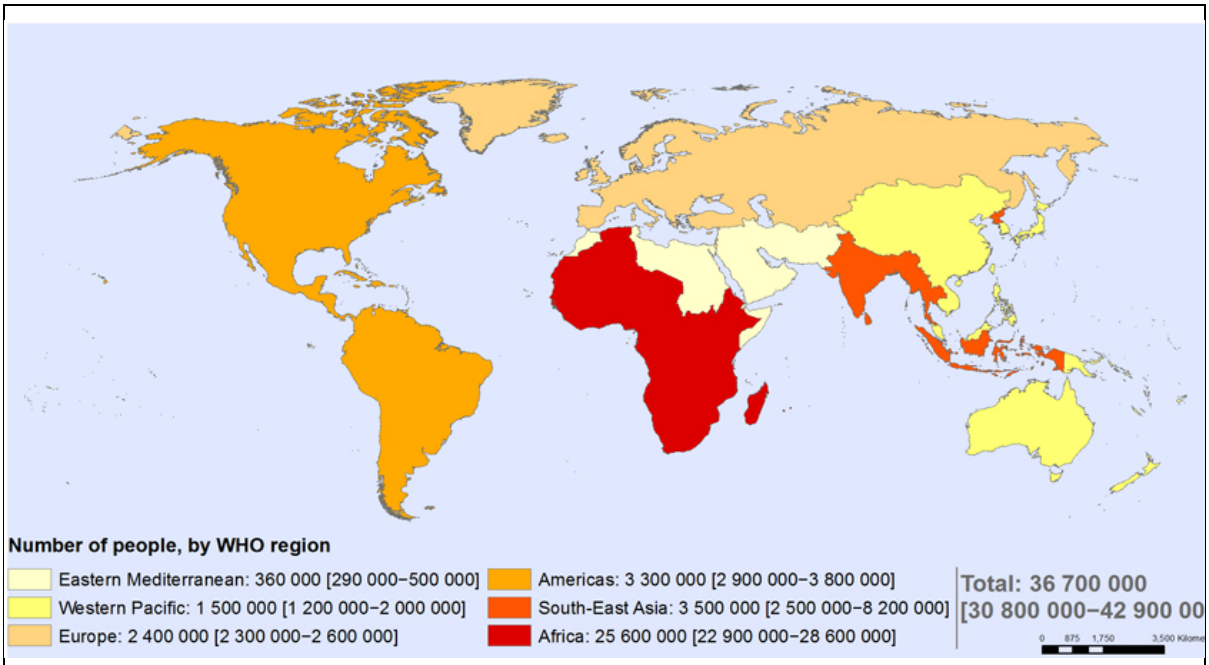

Figure 3-1. *The estimated number of people (all ages) living with HIV in 2016 according to World Health Organisation region. [Image sourced from the World Health Organisation website, http://www.who.int/gho/hiv/epidemic_status/cases_all/en/]*

4 The Current Global Distribution of Tuberculosis

Approximately 4000 people die every day from active tuberculosis, and there was an estimated total of 1.4 million deaths in 2016 across the world (WHO, 2017). There were an estimated 10.4 million new cases of active tuberculosis in 2016, an increase from the estimated 8.6 million new cases in 2012 and the highest number of people with the disease at any point in history (WHO, 2017). However, it must be remembered that many cases of tuberculosis still go undiagnosed, untreated, or unreported and these figures may be significantly under-estimating the true burden of the disease: the upper limit to the estimated incident cases provided by the World Health Organisation (WHO) for 2016 was 12.2 million (WHO, 2017). Central and southern Africa were reported as having some of the highest incidence rates per 100,000 of the population in 2016 (Figure 4-1).

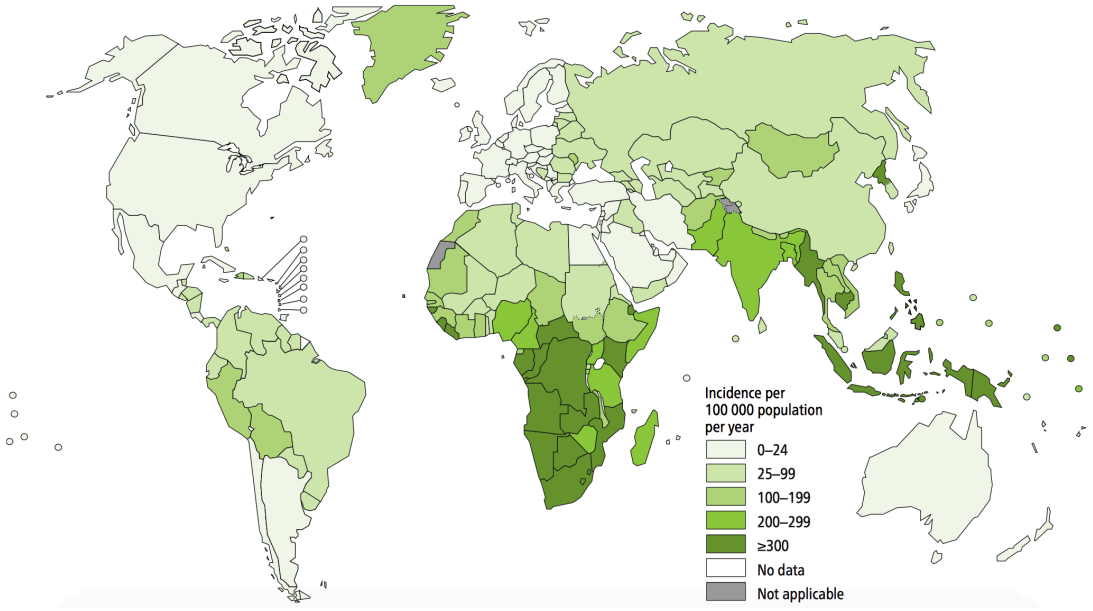

Figure 4-1. *Global estimated TB incidence rates for 2016 sourced from the WHO Global Tuberculosis Report 2017. [WHO. Global Tuberculosis Report. Geneva 2017]*

India, Indonesia, China, the Philippines, and Pakistan are the five countries cited as having the highest burden of disease. In 2016, they accounted for an estimated 56% of the incident cases across the world, and the WHO African region was thought to represent a further 25% of the new presentations (WHO, 2017). India alone was estimated to have had 2,790,000 new cases of tuberculosis in 2016 (WHO, 2017) and, as is the case for the many of the other countries, this information was calculated using case notification data. It should be noted that India's large population of over 1 billion people is a significant factor in its contribution to the number of new tuberculosis cases worldwide, as can be seen by comparing the incidence rate per 100,000 of the population in Figure 4-1 and the total number of incident cases from 2016 in Figure 4-2.

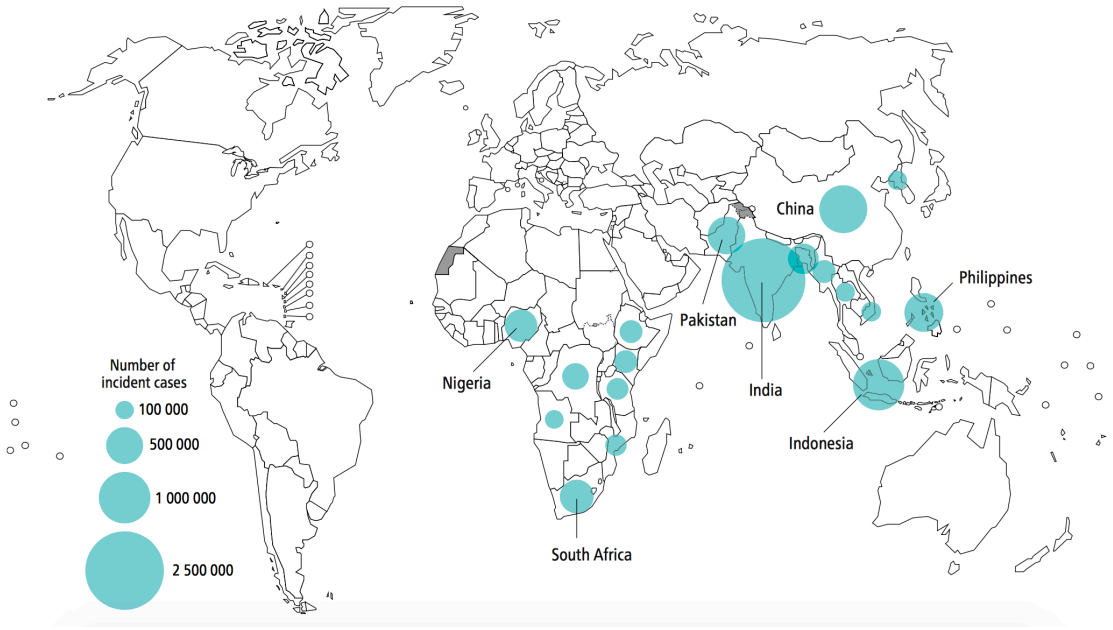

Figure 4-2. *Globally estimated TB incidence in 2016, for countries with at least 100 000 incident cases, sourced from the WHO Global Tuberculosis Report 2017. [WHO. Global Tuberculosis Report. Geneva 2017]*

The emergence of drug-resistant tuberculosis (DR-TB) has been identified as a major factor continuing to drive the ongoing global epidemic, alongside coinfection with HIV (Pawlowski *et al.*, 2012; WHO, 2012; Eldholm *et al.*, 2016; Dheda *et al.*, 2017; Floyd *et al.*, 2018). There were over 600,000 new cases of tuberculosis resistant to rifampicin in 2016, and this number includes a reported 19% of previously-treated cases presenting in 2016 (WHO, 2017). China, India, and the Russian Federation together account for 47% of the DR-TB cases world-wide (WHO, 2017). A failure to sufficiently address these drivers in the tuberculosis epidemic will in turn lead to a failure to end the epidemic in anything approaching the proposed timescale of the World Health Organisation's End TB strategy (WHO, 2017).

Tuberculosis has always been the hallmark disease of vulnerable and marginalised populations, and remains so to this day. Migrant populations, homeless individuals, itinerant people, those in lower socio-economic groups and injecting drug users are all recognised as at-risk groups. This increased risk is due to one or more factors such

as poor nutrition, overcrowding, a lack of adequate sanitation, social deprivation, risk-taking behaviour, stress, smoking, and poor social capital (Aerts *et al.*, 2006; Fleming *et al.*, 2006; WHO, 2017; Aldridge *et al.*, 2018). Sadly, the social circumstances that characterise most of these groups can make the implementation of public health measures and delivery of tuberculosis treatment challenging (Pablos-Méndez *et al.*, 1997; Storla, Yimer and Bjune, 2008). These social circumstances are compounded by the length of treatment and toxicity associated with standard tuberculosis therapy.

5 Toxicity and Adverse Events during Standard TB Therapy

Unwanted side-effects of the medication that have a negative physiological impact on the patient, hereafter referred to as “toxicity”, are a well-recognised complication of standard tuberculosis therapy. Hepatic toxicity, peripheral and optic neuropathies, and gastrointestinal disturbance are just some of the acknowledged side-effects associated with the medication used to treat drug-sensitive pulmonary TB (Forget and Menzies, 2006; Lorent *et al.*, 2011; Zhang *et al.*, 2015; Sarkar, Ganguly and Sunwoo, 2016). Toxicity during therapy is associated with poor adherence, treatment interruptions, and a higher risk of treatment failure (Iseman, 2002; Saukkonen *et al.*, 2006; WHO, 2017); the fall in cure rates from over 90% in trials to approximately 80% in real-world settings (WHO, 2017) is at least partly related to toxicity from standard tuberculosis therapy. Rapid identification of adverse reactions to medications has been shown to improve outcomes (Gholami *et al.*, 2006; Marra *et al.*, 2007; Lehloenya and Dheda, 2012; Michail, 2013; Naik *et al.*, 2015) as the offending agent can be withheld and appropriate treatment can be implemented. Therefore, it is crucial that tuberculosis services are set up to identify patient groups at the highest risk of a

complicated course on treatment and are also aware of the most common patterns of toxicity to optimise the management of their patients.

The majority of the published work on clinical implications of toxicity relating to standard tuberculosis therapy is retrospective or observational in nature and involves small numbers of patients, and as such suffers from a number of limitations. As is the case with retrospective and observational studies, there is a risk of both observed and unknown bias and confounding affecting the results through the sampling of the study population. There are some studies available, predominantly relating to hepatotoxicity (Kumar *et al.*, 2010; Shang *et al.*, 2011; Shu *et al.*, 2013; Yimer *et al.*, 2014), that have large numbers of patients involved in the analysis and this may attenuate the risk of confounding (although this could actually be accentuated through selection bias), but they still rely on passive detection of significant toxicity or a review of patient notes.

Conclusions drawn from the literature are further impaired by the varying definitions employed for “toxicity”; for example, the quoted rates of hepatotoxicity attributed to standard TB therapy range from 5-33% (Thompson *et al.*, 1995; Tost *et al.*, 2005; Saukkonen *et al.*, 2006; Tostmann *et al.*, 2008; Kumar *et al.*, 2010) in published reports. A large part of the explanation for this variation lies in some studies including only cases with associated clinical symptoms, varying laboratory thresholds to define “hepatotoxicity”, or different periods of observation for patients. The tuberculosis community has therefore continued to operate in quite a reactive manner to treatment complications, aware of common toxicities but with no clearly defined guidance on timing, risk factors, clinical course, or what the best evidence-based approach is to the management of side-effects.

5.1 Toxicity Associated with Drugs used for Tuberculosis Treatment

The drugs used in the treatment of active tuberculosis are never given in isolation, due to the risk of resistance, and therefore it is not easy to make definitive statements about their individual side effects. Additionally, there could be differences in the drug dynamics in healthy volunteers compared to patients with active TB disease. Isoniazid and rifampicin are used in isolation as treatment for latent tuberculosis infection (Akolo *et al.*, 2010; Sharma *et al.*, 2014), and moxifloxacin is used alone in the treatment of other bacterial infections (Grossman *et al.*, 2014), and this has allowed for the collection of some clinical evidence for individual drug toxicity. Data relating to the drugs in other diseases, basic science work looking at *in vitro* activity of the drugs, early clinical trials and observational work can be used in combination to gain some understanding of the expected toxicity associated with each medication and the mechanisms responsible.

5.1.1 Isoniazid Toxicity

Isoniazid is a prodrug with an active metabolite (nicotinoyl-NAD) that acts to inhibit mycolic acid synthesis, which is an essential component of the mycobacterial cell wall. The prodrug is metabolised by a catalase-peroxidase enzyme in *M. tuberculosis* called KatG, and the active metabolite eventually blocks the activity of the protein reductase InhA which finally leads to impaired activity of fatty acid synthase(Timmins and Deretic, 2006). Isoniazid predominantly acts as a bactericidal agent against actively dividing mycobacteria, killing almost 90% of mycobacteria in a case of active tuberculosis within a matter of days (Donald and Diacon, 2008), but also exerts a bacteriostatic effect (as well as bacteriocidal) on dormant organisms (Heifets, Lindholm-Levy and Flory, 1991).

Hepatotoxicity is a well-recognised side effect of isoniazid use and the quoted rates range between approximately 0.1% to 20% (Nolan, Goldberg and Buskin, 1999; Grant *et al.*, 2010; Al-Darraji, Kamarulzaman and Altice, 2012; Hassan *et al.*, 2015; Metushi, Uetrecht and Phillips, 2016). In the human host, isoniazid is metabolised in the liver through acetylation by N-acetyl transferase 2 (NAT2) to produce acetylisoniazid (Cordes *et al.*, 2016). Figure 5-1 summarises the metabolic pathway for isoniazid in the liver. Polymorphisms of NAT2 are used to categorise patients as either slow- or fast-acetylators. The former produces hydrazine predominantly when greater doses of isoniazid are administered, while the latter will produce higher levels of acetylhydrazine, and these are both toxic metabolites that damage liver tissue through oxidative stress (Perwitasari *et al.*, 2015; Metushi, Uetrecht and Phillips, 2016; P. Wang *et al.*, 2016). Therefore, the cause of isoniazid-induced hepatotoxicity is thought to be slightly different depending on the patient's acetylator status, although ultimately resultant from oxidative stress effects on hepatocytes due to hydrazine generation.

As can also be seen in Figure 7, CYP2E1 also plays an important role in the generation of oxidised hepatotoxic hydrazines. CYP2E1 polymorphisms have been shown to influence the risk of tuberculosis treatment-associated liver injury, both in isolation and in conjunction with NAT2 genotypes (Vuilleumier *et al.*, 2006; Sheng *et al.*, 2014; Singla *et al.*, 2014). In light of this, the optimal dosing strategy is still unclear, whereby antimicrobial activity is maximised with minimal risk of hepatotoxic effects based on the presence or absence of individual genetic polymorphisms (Huang *et al.*, 2002; Azuma, Ohno and Kubota, 2013; Cordes *et al.*, 2016; Toure *et al.*, 2016).

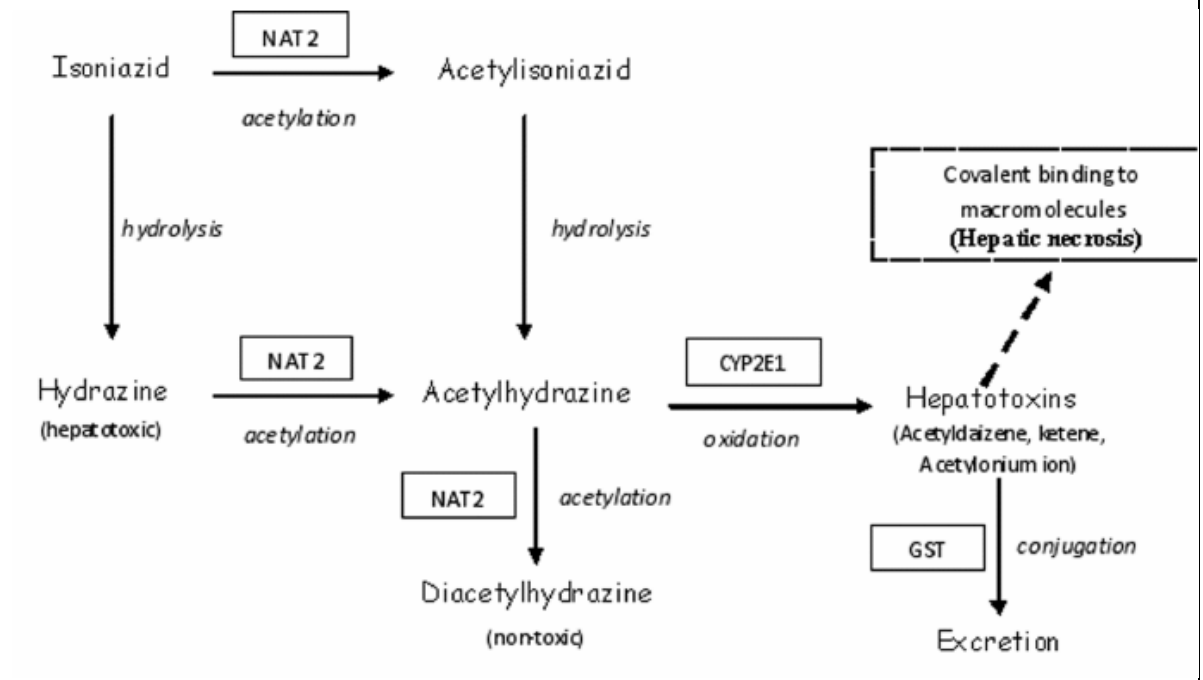

Figure 5-1. *Flow diagram illustrating hepatic metabolism of isoniazid. Isoniazid is metabolised to acetylhydrazine by one of two pathways regulated by the NAT2 enzyme, and "slow" or "fast" acetylators will predominantly accumulate the toxic metabolites hydrazine or acetylisoniazid, respectively. Acetylhydrazine is converted to the non-toxic diacetylhydrazine by NAT2 in the liver preferentially but it is also oxidised into hepatotoxic metabolites. Enzymes involved in the process are included in boxes above the arrows linking metabolites and the metabolic process is described in italics. NAT2= N-acetyl transferase 2; CYP2E1= Cytochrome P4502E1; GST= Glutathione S-transferase. Imaged reproduced from "Tuberculosis – Current Issues in Diagnosis and Management", Chapter: Tuberculosis Pharmacogenetics: State of the Art, P109 (Mahboub and Vats, In Tech – Open Science, 2013)*

Peripheral neuropathy affects between 2% and 10% of patients taking isoniazid on average(Kass and Shandera, 2010). It is mediated by isoniazid inhibiting the activation of pyridoxine to essential co-enzymes for protein metabolism and the production of some neurotransmitters. The risk of neuropathy is increased with HIV infection, alcohol abuse, diabetes, malnourishment, increasing age, and with concurrent use of other medications that antagonise the effects of vitamin B6 (e.g. hydralazine, cycloserine) (Desai and Agarwal, 2017). Isoniazid overdose is also associated with seizure activity (Kass and Shandera, 2010).

There are other less common side effects of isoniazid. Rhabdomyolysis can result from a direct toxic effect of isoniazid metabolites on skeletal muscle or secondary to prolonged seizure activity (Blowey, Johnson and Verjee, 1995; Panganiban,

Makalinao and Cortes-Maraba, 2001). Agranulocytosis is a very rare and idiosyncratic drug reaction from isoniazid use, and the effect seems to reverse within days of stopping the drug (Claiborne and Dutt, 1985; Bidarimath *et al.*, 2016). Isoniazid can also cause a pyridoxine-responsive sideroblastic anaemia (Morrow *et al.*, 2006). In cases of isoniazid overdose there is infrequently a severe metabolic acidosis caused by a combination of increased lactic acid production during seizures, acidic metabolites of isoniazid, and a ketoacidosis from enhanced fatty acid oxidation (Brown, 1972; Hankins *et al.*, 1987). Finally, there are scattered reports of pancreatitis, febrile reactions, and dermatitis associated with isoniazid use (Desai and Agarwal, 2017).

5.1.2 Rifampicin Toxicity

Rifampicin is a bactericidal drug acting on actively dividing mycobacteria, but it also exerts a bactericidal effect on mycobacteria in a dormant state (Wehrli, 1983). The bactericidal effect of rifampicin is critical to treating pulmonary tuberculosis with six months of standard therapy (Grosset, 1978), and the resistance to rifampicin that is characteristic of multi-drug resistant tuberculosis is a large part of why that infection is so difficult to treat. Rifampicin acts on *M. tuberculosis* by inhibiting DNA-dependent RNA polymerase, interfering with the organism's gene transcription and the synthesis of messenger RNA, and this eventually leads to cell death (Wehrli, 1983).

Hepatitis presenting with a cholestatic picture on liver biochemical tests occurs in approximately 1% to 3% of patients being treated with rifampicin in isolation (Lardizabal *et al.*, 2006; Menzies *et al.*, 2008; Fountain *et al.*, 2009; Ziakas and Mylonakis, 2009). An asymptomatic elevation in serum bilirubin without hepatic enzyme elevation is seen in approximately 5% of patients receiving rifampicin in isolation (Grosset and Leventis, 1983; Byrne *et al.*, 2002). Over 80% of the ingested

drug is metabolised in the liver through the CYP450 enzymatic system, and the drug is excreted via the biliary tract (Acocella, 1983). The hepatic metabolism of rifampicin leads to an upregulation of the CYP450 enzymes in the liver, and this induction can lead to reduced concentrations of other drugs that are metabolised through the same pathway. Drugs that are especially affected are some anti-retroviral therapies, oral contraceptives, warfarin, and clarithromycin with a consequent reduction in the efficacy (Niemi *et al.*, 2003).

Rifampicin has a relatively favourable toxicity profile in the existing literature, and other side-effects are only infrequently reported. Immunological reactions causing blood dyscrasia, autoimmune thrombocytopenia, vasculitis, haemodynamic shock, and interstitial nephritis have been identified in less than 0.1% of patients taking rifampicin (Mary Aquinas *et al.*, 1972; Girling, 1977; Grosset and Leventis, 1983; Jung *et al.*, 2007; Mori *et al.*, 2011; Chiba *et al.*, 2013). These immunological reactions, in particular interstitial nephritis, have been observed more frequently in patients taking rifampicin intermittently compared to those taking the drug on a daily basis (Grosset and Leventis, 1983). Exanthema has been noted due to rifampicin use and generally resolves when the drug is withdrawn (Lehloenya and Dheda, 2012). Mild gastrointestinal upset can also occur, but this rarely leads to an interruption in therapy (Forget and Menzies, 2006).

5.1.3 Pyrazinamide Toxicity

Pyrazinamide is a nicotinic acid derivative first synthesised in 1936 and has been used as an anti-tuberculosis drug since as early as 1952 (Fox, Ellard and Mitchison, 1999). The drug is bactericidal and has a powerful sterilising effect in the treatment of tuberculosis infection (Grosset, 1978; Steele and Des Prez, 1988; Zhang and Mitchison, 2003). The drug is initially absorbed into the mycobacteria and then

subsequently converted to the active metabolite pyrazinoic acid by the enzyme pyrazinamidase (encoded by the *pncA* gene) (Momekov *et al.*, 2014) and is also activated by human hepatic amidases (Laine *et al.*, 1989). Pyrazinoic acid then diffuses out of the mycobacteria and, in the acidic conditions found in the granuloma, is converted to a conjugate acid which then diffuses back into the mycobacteria again and accumulates (see Figure 5-2). The precise mechanism by which the pyrazinoic acid exerts its bactericidal effect is unknown at the time of writing, and possibly involves inhibition of fatty acid synthase (Zhang and Mitchison, 2003; Momekov *et al.*, 2014).

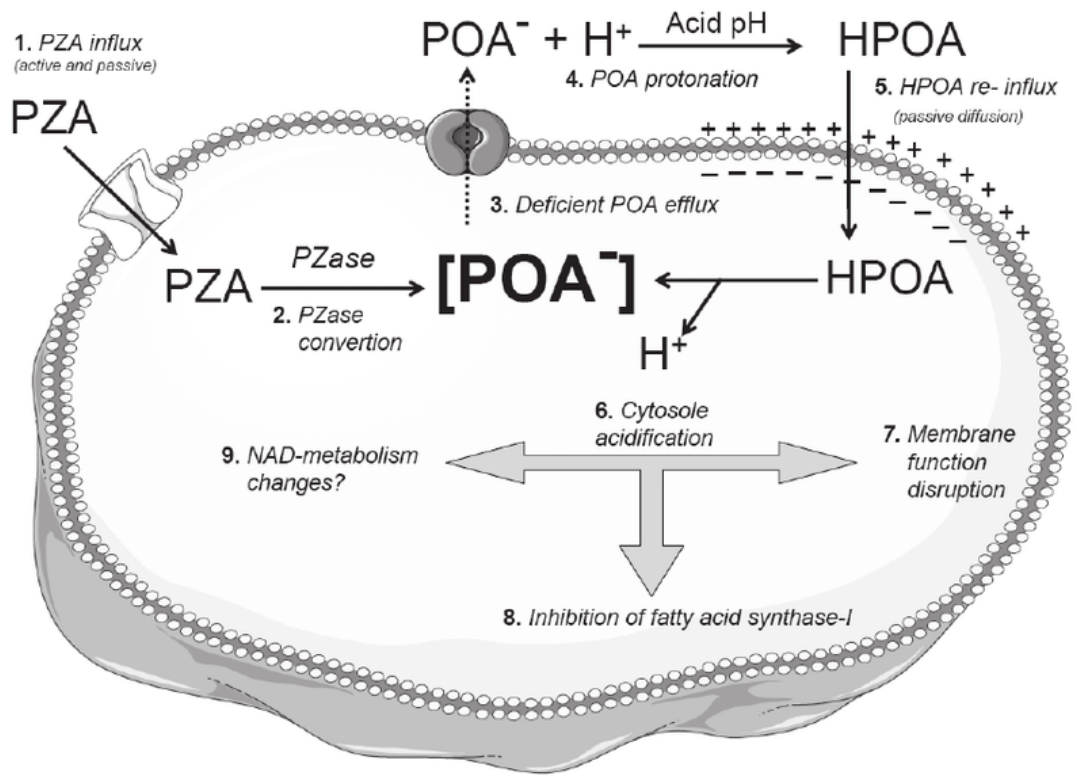

Figure 5-2. *Diagrammatic representation of pyrazinamide metabolism within M. tuberculosis. Pyrazinamide enters the mycobacteria through passive diffusion and is converted to pyrazinoic acid by pyrazinamidase. Pyrazinoic aid is then excreted from the cell via a weak efflux pump and converted to protonated pyrazinoic acid by hydrogen-donation from the acidic environment. This protonated pyrazinoic acid is reabsorbed into the mycobacteria, where it begins to accumulate due to the inefficiency of the efflux pump and causes cellular damage through one or more proposed mechanisms. PZA= Pyrazinamide; POA= Pyrazinoic acid; PZase= Pyrazinamidase; HPOA= Protonated POA. Image reproduced from Momekov G, Ferdinandov D, Voynikov Y et al. Pyrazinamide – Pharmaceutical, biochemical and pharmacological properties and reappraisal of its role in the chemotherapy of tuberculosis. Pharm 2014;61(4):38-67(Figure 2 in article).*

Liver toxicity is an established side effect of pyrazinamide use in tuberculosis treatment (Durand *et al.*, 1995; Saukkonen *et al.*, 2006; Chang *et al.*, 2008; Shih *et al.*, 2013; Jeong *et al.*, 2015). The drug is predominantly metabolised in the liver (Donald and McIlleron, 2009), and hepatotoxicity was observed in the early studies where patients were treated with pyrazinamide (Angel, Somner and Citron, 1979). The hepatotoxicity can be either dose-related, as was seen in the early dosing studies, or idiosyncratic at accepted treatment doses (Chang *et al.*, 2008; Pasipanodya and Gumbo, 2010; Sahota and Pasqua, 2012). Pyrazinamide-induced hepatotoxicity can present in a sub-acute manner and is often severe (Hussaini and Farrington, 2007; Yew and Leung, 2007). The mechanism of pyrazinamide-mediated hepatotoxicity is

not clear, but the metabolite 5-hydroxypyrazinoic acid has been implicated (Momekov *et al.*, 2014).

The efficacy of pyrazinamide in treating pulmonary tuberculosis is matched by its side-effect profile, and it is generally considered to be the most toxic of the four drugs used in standard tuberculosis therapy. Pyrazinoic acid inhibits the renal secretion of uric acid and this causes increased serum uric acid levels (Donald and McIlleron, 2009). Acute arthritis can occur in the context of drug-induced hyperuricaemia, and those patients with a previous history of gout are at increased risk (Jenner *et al.*, 1981). Rhabdomyolysis is a rare but severe complication of pyrazinamide use, possibly due to a toxic effect exerted on cell mitochondria, and this can lead to renal impairment in some cases (Arbex *et al.*, 2010). Additionally, pyrazinamide can cause a photosensitivity dermatitis in some patients that resolves after withdrawal of the medication (Bharat *et al.*, 2003).

5.1.4 Ethambutol Toxicity

Prevention of the development of resistance is the primary reason for including ethambutol in standard tuberculosis therapy (WHO, 2011b; Nahid *et al.*, 2016). It is a synthetic compound that has been in use since 1966 for the treatment of TB (Fox, Ellard and Mitchison, 1999). Ethambutol works to interfere with mycobacterial cell wall synthesis by inhibiting the action of the *embB* gene which is responsible for the polymerisation of arabinose into arabinogalactan within the mycobacteria (Forbes, Kuck and Peets, 1962). This is an essential component of the mycobacterial cell wall, and ethambutol exerts a bacteriostatic effect on actively dividing mycobacteria through the inhibition of this polymerisation reaction. The drug is metabolised in the liver into dicarboxylic acid via an intermediate aldehyde but there is no change in the activity of the liver enzymes (Forbes, Kuck and Peets, 1962).

Ethambutol has the most favourable toxicity profile of the four drugs used in standard TB therapy, and side effects are generally dose-related (Sarkar and Ganguly, 2016). Bilateral optic neuropathy is a serious toxic effect of ethambutol and most commonly presents with a retrobulbar neuritis affecting the axial fibres, likely related to mitochondrial dysfunction (Fraunfelder, Sadun and Wood, 2006). The central fibres are more often affected than the peripheral fibres and this manifests clinically as reduced visual acuity, impaired colour vision, and scotomas (Lim, 2006). The overall risk of developing optic neuropathy is thought to be less than 1% at doses less than 15mg/kg body weight per 24 hours (Ezer *et al.*, 2013; Kim and Park, 2016; Yang *et al.*, 2016). The risk is increased in the context of renal disease, due to increased serum concentrations, but the effects are generally reversible if the drug is stopped promptly (Fraunfelder, Sadun and Wood, 2006).

Ethambutol can also rarely cause a peripheral neuritis in cases when vitamin B2 levels are low (e.g. older age or alcohol excess) (Kass and Shandera, 2010). Other toxic effects of the drug are either mild in nature, very rare, or both, and the literature relating to them predominantly takes the form of isolated case reports: these include gastrointestinal upset, depression, blood dyscrasia, hypersensitivity reactions and arthropathy secondary to uric acid retention (Kwon *et al.*, 2004; Lehloenya and Dheda, 2012; Yen *et al.*, 2015; Kassa *et al.*, 2016).

5.1.5 Moxifloxacin Toxicity

Moxifloxacin is an 8-methoxy-fluoroquinolone that was first licenced in 1999 for the treatment of a range of bacterial infections including community-acquired pneumonia, skin and soft tissue infections, and intra-abdominal infections (Gillespie, 2016). In the late 1990s and early 2000s there was increasing interest in the drug's ability to treat tuberculosis, and this led to early-phase clinical studies that demonstrated potent

bactericidal activity against *M. tuberculosis* (Ji *et al.*, 1998; Nuermberger *et al.*, 2004). Moxifloxacin is used in cases where patients are unable to tolerate standard tuberculosis therapy and in the treatment of drug-resistant tuberculosis (Gillespie, 2016). It exerts a bactericidal effect by inhibiting the activity of DNA gyrase with a consequent disruption of cell replication (Donald and McIlleron, 2009).

The drug is metabolised in the liver to an N-sulfate conjugate and an acyl glucuronide which are then excreted in the urine and faeces, but urinary excretion of unchanged drug accounts for approximately 20% of the given dose (Sullivan *et al.*, 1999; Moise, Birmingham and Schentag, 2000). Moxifloxacin does not act to alter hepatic enzyme activity, but co-administration with rifampicin can lead to reduced plasma concentrations of moxifloxacin (Nijland *et al.*, 2007). Approximately 1 – 10% of patients may experience an asymptomatic elevation in liver enzymes while taking the drug (Bertino and Fish, 2000; Ball *et al.*, 2004), and there are very rare reports of fulminant liver failure mediated by a hypersensitivity reaction in hepatic tissue (Soto *et al.*, 2002; Nori *et al.*, 2004; Verma *et al.*, 2009).

Moxifloxacin has a generally favourable side effect profile, but cardiac toxicity and tendonitis are the two other rare toxicities that have been reported. Blockade of the cardiac potassium channels can cause delayed repolarisation and prolongation of the QT interval occurs in 1-10% of cases (Haverkamp *et al.*, 2012; Mehrzad and Barza, 2015). Cardiac arrest with either unspecified arrhythmias or torsade de pointes is thought to affect less than 0.01% of patients, however (Owens and Nolin, 2006; Altin *et al.*, 2007; Briasoulis, Agarwal and Pierce W J, 2011). It is estimated that approximately 0.01 – 0.1% of patients will experience tendinopathy that is believed to be mediated through ischaemia with only a very small proportion rupturing tendons as a result (van der Linden *et al.*, 2002; Khaliq and Zhanel, 2003). Other potential side

effects with a risk of approximately 0.01 – 0.1% include coagulopathy, agranulocytosis, significant electrolyte disturbance, psychiatric symptoms, and drug-induced nephropathy (Food and Drug Association, 1999; Ball *et al.*, 2004).

6 Further Work to Shorten Tuberculosis Treatment

6.1 The Climate in Tuberculosis Research at the end of the 20th Century

After decades of active research output, transforming tuberculosis from a largely fatal and incurable condition into one with an effective treatment, the BMRC closed its doors in 1986. Trial after trial had created a robust framework for TB surveillance, treatment regimens, and supervision of therapy and now the bulk of the work rested on the public health authorities to implement these in control programmes. However, an inability to bring the TB epidemic under control in low and middle income countries made it apparent that it was not as simple as that. Among other things the HIV epidemic, living conditions associated with poverty, and emerging drug resistant strains all worked together to undermine efforts to eradicate tuberculosis in these regions.

Standard tuberculosis therapy also did not perform as well outside of controlled trial conditions and the efficacy of treatment fell from over 90% in the trial setting (Fox, Ellard and Mitchison, 1999) to approximately 80-85% in the field (WHO, 2017). Toxic side effects, lengthy duration of treatment, limited infrastructure, an improvement in symptoms with reduced drive to adhere, chaotic lifestyles, and social stigma relating to a tuberculosis diagnosis are just some of the reasons that standard tuberculosis therapy becomes less effective in real-world settings. At the end of the 20th century a renewed interest started to gather pace towards reducing the treatment duration to less than 6 months for drug-sensitive tuberculosis; if the treatment duration could be

lessened without compromising efficacy then a major barrier to adherence would be removed and real-world cure rates could be brought closer to 90%.

6.2 The Fluoroquinolone Studies

At the beginning of this century murine models and early phase clinical studies demonstrated potent activity of moxifloxacin against *M. tuberculosis* (Gosling *et al.*, 2003; Pletz *et al.*, 2004; Rustomjee *et al.*, 2008). The potential for moxifloxacin to shorten the duration of treatment for tuberculosis was reinforced by clinical studies investigating the early bactericidal activity at 8 weeks of regimens with fluoroquinolones substituted for isoniazid or ethambutol (Burman *et al.*, 2006; Rustomjee *et al.*, 2008; Conde, Efron, Loredo, Muzy, *et al.*, 2009; Dorman *et al.*, 2009). There was also now more of a willingness on the part of major funding bodies to engage with tuberculosis research, and this led to three phase III trials investigating the impact of fluoroquinolones on the treatment of pulmonary tuberculosis launching in the first decade of the 2000s. All three of these trials sought to investigate the efficacy of at least one four-month, fluoroquinolone-containing treatment regimen for drug-sensitive pulmonary tuberculosis.

6.2.1 The OFLOTUB Study

This study sought to determine if gatifloxacin could be substituted into standard tuberculosis therapy to allow the treatment duration to be reduced from 6 to 4 months (Gninafon *et al.*, 2014). The experimental arm in the trial consisted of standard tuberculosis therapy with gatifloxacin substituted for ethambutol in the intensive phase, and then given alongside isoniazid and rifampicin for a further two months for a total of 4 months' treatment. Over 1800 patients were recruited from five sub-Saharan countries and randomised to receive either standard tuberculosis therapy or the experimental regimen between 2005 and 2009. Patients were to be followed for

24 months after treatment completion and a non-inferiority design was used to assess the primary efficacy endpoint of an unfavourable outcome on treatment (treatment failure, recurrence, death, or study dropout during treatment). A non-inferiority margin of 6 percentage points at the upper bound of the difference for unfavourable outcomes, adjusted for country, was agreed during the design of the study.

The risk of an unfavourable outcome was 17.2% with standard tuberculosis therapy and 21.0% on the experimental arm in the modified intention-to-treat analysis, and the adjusted difference was 3.5 percentage points (95% confidence interval [CI], -0.7 to 7.7). Therefore, non-inferiority was not achieved by the experimental arm based on the primary endpoint. In other aspects, standard tuberculosis therapy did not perform as well as the gatifloxacin-containing arm with a higher dropout rate during treatment (5.0% vs 2.7%) and more treatment failures (2.4% vs 1.7%). Despite this, however, there were fewer recurrences on standard tuberculosis therapy (7.1% vs 14.6%).

There were similar numbers of Serious Adverse Events (SAEs) reported on the control (23 events) and experimental (20 events) arms. On the standard tuberculosis therapy arm 10 of 23 (43.5%) SAEs were related to respiratory symptoms and 4 of 20 (20.0%) SAEs on the experimental arm were related to diabetes or meningitis (grouped under “nervous system disorders”). In particular, there was no increased risk of QT interval prolongation or dysglycaemia for patients taking the experimental regimen.

6.2.2 The RIFAQUIN Study

The RIFAQUIN trial group built on murine models that suggested combining high-dose rifapentine with a fluoroquinolone would overcome the heightened risk of relapse or acquired resistance seen previously with intermittent dosing (Jindani *et al.*, 2014). This non-inferiority study involved two experimental regimens: both replacing isoniazid with moxifloxacin given daily during the intensive phase, followed by moxifloxacin and

rifapentine administered twice per week for a further two months or in one weekly dose for four months. There were 827 patients enrolled from South Africa, Zimbabwe, Botswana, and Zambia. A primary end point of treatment failure or relapse was used to compare the experimental arms to standard tuberculosis therapy, with non-inferiority set at a margin of 6 percentage points difference using 90% confidence intervals.

The six-month experimental arm in the trial met the criteria for non-inferiority compared to standard tuberculosis therapy. On the control arm, 14.4% of patients had an unfavourable outcome, compared to 13.7% on the six-month arm in the modified intention-to-treat analysis (adjusted difference 0.4 percentage points; 90% CI, -4.7 to 5.6). Once more, reducing the treatment duration to four months meant non-inferiority was not achieved with 26.9% of patients classed as unfavourable (adjusted difference 13.1 percentage points; 90% CI, 6.8 to 19.4).

There were 45 adverse events that were graded as severe or life-threatening during the trial. Across the three treatment arms there were 16, 12, and 17 grade 3 or 4 adverse events reported on the standard tuberculosis therapy, 4-month, and 6-month regimens respectively. There were 6, 12, and 7 deaths reported on the standard, 4-month, and 6-month arms. Among the 25 deaths only 4 were assessed as being possibly or probably related to the trial medication, and 8 deaths did not have a clear cause reported (all had negative sputum culture prior to death, and 6 were HIV co-infected).

6.2.3 The REMoxTB Study

The Rapid Evaluation of Moxifloxacin in Tuberculosis (REMoxTB) study sought to evaluate the efficacy and safety profile of two experimental treatment regimens compared to standard tuberculosis therapy (Gillespie *et al.*, 2014). Patients were

recruited into the randomised, controlled trial over a period of 6 years and allocated to one of three treatment arms: standard tuberculosis therapy as a control arm, or a four-month experimental arm with moxifloxacin substituted for either ethambutol (the "isoniazid arm") or isoniazid (the "ethambutol arm"). The trial used a non-inferiority design to assess the efficacy of the two experimental arms compared to standard tuberculosis therapy after almost 2000 patients completed 18 months of follow-up. Non-inferiority was defined as a between-group difference of less than 6 percentage points in the upper boundary of the two-sided 97.5% Wald confidence interval for the proportion of patients considered to have an unfavourable outcome. Unfavourable outcomes were defined as an extension in treatment beyond that permitted in the protocol, failing to achieve sustained sputum culture negative status, or death from any cause). The full criteria for favourable and unfavourable outcomes, and for inclusion in analysis populations, can be found in Section 1.6, Chapter 2 of the thesis.

Neither of the experimental arms met the criteria for non-inferiority in the per-protocol population. The relapse-free cure rates were 85% and 80% for the isoniazid and ethambutol arms, and 92% among patients allocated to standard tuberculosis therapy. The adjusted absolute difference in the proportion of patients with an unfavourable outcome compared to standard tuberculosis therapy was 6.1 (97.5% CI, 1.7 to 10.5) and 11.4 percentage points (97.5% CI, 6.7 to 16.1) for the isoniazid and ethambutol arms, respectively.

There was no significant difference detected for the incidence in grade 3 or 4 adverse events across the three treatment arms in the original analysis. There were 123 (19%), 127 (19%), and 111 (17%) patients affected by one or more grade 3 or 4 adverse event on the standard, isoniazid and ethambutol arms. The number of SAEs, type of events, and numbers of patients with SAEs were similar in the three trial arms during

treatment and follow up. Additionally, there was no difference between the groups for incidence of adverse events particularly related to the substitution of fluoroquinolones: cardiac toxicity, tendinopathy, seizures, dysglycaemia, or peripheral neuropathy.

7 Toxicity Related to Tuberculosis Treatment: The Aim of the Thesis

While the REMoxTB study did not result in the adoption of a novel four-month treatment for drug-sensitive pulmonary tuberculosis a significant advance that came from the trial was the collection of stringent, prospectively-gathered data relating to 639 patients receiving standard tuberculosis therapy (Gillespie *et al.*, 2014). Microbiological, clinical, demographic, and safety data was incorporated into the database for every patient randomised into the trial and, where possible, even for patients who were withdrawn from treatment and referred to the National Treatment Program. The result is a rich database with the potential to provide firm answers to clinical questions that previously have been answered through a combination of anecdote or retrospective and observational studies prone to bias and confounding.

There are many reasons why our efforts to control the global tuberculosis epidemic have struggled to curb the disease at a rate in line with policy timelines (Floyd *et al.*, 2018), and the toxicity associated with standard tuberculosis therapy is one of them. At this point in time, standard tuberculosis therapy is unlikely to be replaced with a novel regimen anytime soon (Laughon and Nacy, 2017) and there is a need to better equip tuberculosis physicians with an evidence-based approach to managing treatment toxicity. Improved monitoring strategies based on robust data, fuelled by increased funding attracted through clearly-defined effects on treatment outcome from toxic events, could help move cure rates towards 90% and reduce the disease burden.

The effort to shorten tuberculosis treatment to less than 6 months continues despite the disappointing results of the fluoroquinolone trials. The drive to shorten therapy is based on the logistic difficulties in administering lengthy treatment, poor adherence due to treatment duration, and reducing toxicity through reduced exposure (Pai *et al.*, 2016). Despite failing to achieve the pre-defined non-inferiority criteria in the study, both of the experimental arms in REMoxTB demonstrated similar relapse-free microbiological cure rates to those seen with standard tuberculosis therapy outside of trial settings (Gillespie *et al.*, 2014). Therefore, we have two four-month treatment regimens that could be as effective as standard tuberculosis therapy in real world settings as they are less complex to administer, and with similar adverse event incidence in the original analysis. However, this would need to be confirmed with further prospective work as it could be that the experimental arms actually have a reduced efficacy in the field compared to their performance in trial conditions (similar to existing standard TB therapy).

The aim of this thesis is to use the data made available through the REMoxTB database to address two broad questions. First, how toxic is standard tuberculosis therapy? Specifically, to determine which patient groups are at highest risk, when toxicity occurs, and what form it most commonly takes. This can be coupled with an analysis of the impact on treatment outcome using stringently gathered microbiological data to produce recommendations for monitoring practice and build an argument supporting the direction of resources towards this program. Second, how does this toxicity profile compare to that seen on the four-month treatment arms in REMoxTB? An accurate characterisation of the toxicity and adverse events observed with patients allocated to the experimental arms may help define a place for them in the management of pulmonary tuberculosis. The future of tuberculosis treatment should

not be a single new regimen but rather a choice of treatment options that will allow us to treat the disease in a tailored way (accounting for patient characteristics such as personalised risk of toxicity, environmental factors such as ability to perform safety monitoring, and resistance patterns of the mycobacteria causing disease). The goal is to minimise duration and toxicity, and maximise efficacy and a detailed analysis of these experimental arms could bring us closer to this point.

Tuberculosis continues to claim thousands of lives every day, and the fight against it is far from over. A more informed approach to the management of side-effects from standard tuberculosis therapy would help with adherence and more can be made of the treatment that we already have available, while work continues on developing shorter effective regimens. This thesis will present analyses relating to the general pattern of toxicity seen on standard therapy, and in particular hepatotoxicity, and compare this to the patterns seen on the experimental arms. Finally, the course over treatment and toxicity profile seen among the HIV-positive patients receiving standard tuberculosis therapy will be analysed in detail.

Chapter Two: Materials and Methods

1 The REMoxTB Study

The analyses presented in this thesis were all performed using the REMoxTB study database (Gillespie *et al.*, 2014) [Clinicaltrials.gov trial number NCT00864383], and the original New England Journal of Medicine publication can be found in Appendix 1. The Rapid Evaluation of Moxifloxacin in Tuberculosis (REMoxTB) study was a randomised, blinded, placebo-controlled trial comparing two experimental, moxifloxacin-containing four-month treatment arms to standard TB therapy. Human immunodeficiency virus (HIV) positive and HIV-negative patients with sputum smear-positive, drug-sensitive pulmonary TB were recruited between 2007 and 2012.

The study screened 2763 patients and randomised 1931 patients to receive treatment. Patients failed screening most commonly because of sputum smear-negative results, a CD4+ count of less than 250 cells/mm^3 if HIV-positive, or multidrug resistant TB detected using the Hain test. There were 909 patients randomised in South Africa, 376 in India, 212 in Tanzania, 136 in Kenya, 119 in Thailand, 69 in Malaysia, 66 in Zambia, and 22 in both China and Mexico.

1.1 REMoxTB Hypothesis and Design

REMoxTB was designed to investigate the hypothesis that replacing either isoniazid or ethambutol in standard TB therapy with moxifloxacin and treating for four months would result in rates of relapse-free cure that were non-inferior to those seen with six

months of treatment with standard TB therapy. The primary outcome was a non-inferiority comparison of the rates of relapse-free cure between standard TB therapy and the experimental arms individually, and efficacy and safety data was collected for all patients. The trial schematic can be seen in Figure 1-1.

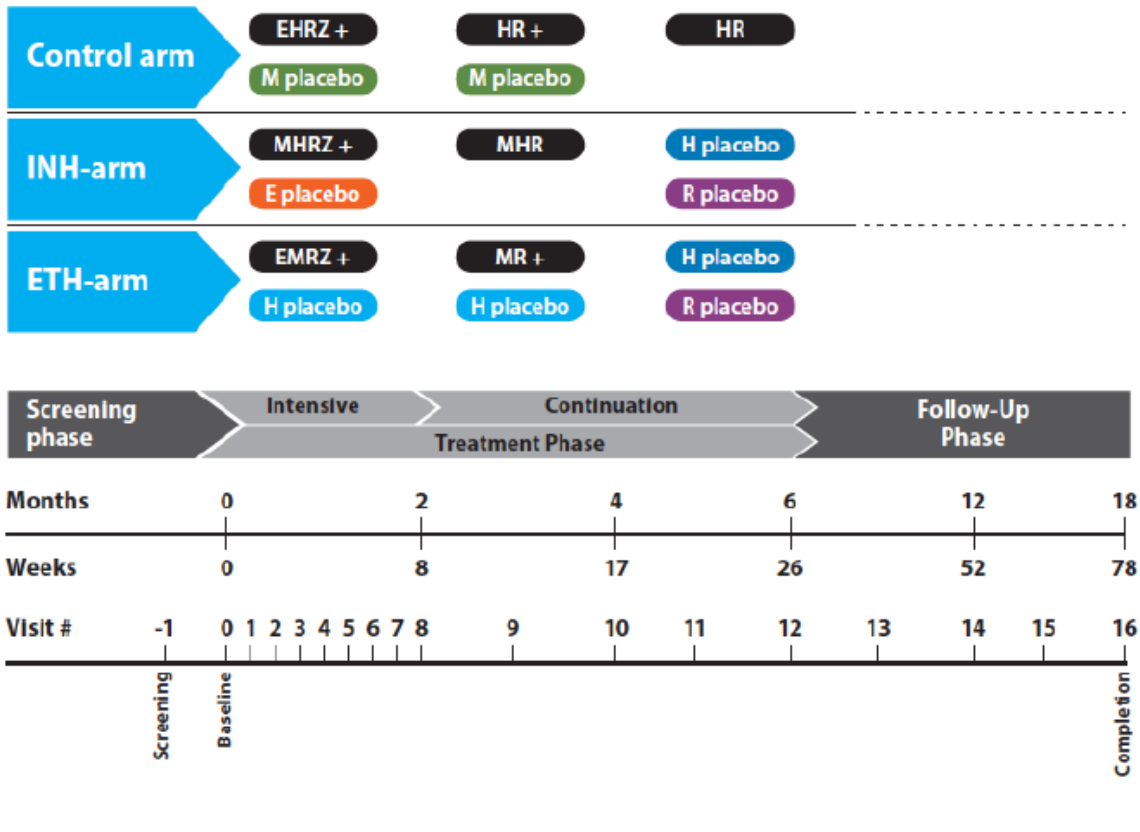

Figure 1-1. *REMoxTB trial schematic describing the regimens and visit schedule. [Obtained from Supplementary Appendix, available at https://www.nejm.org/doi/full/10.1056/NEJMoa1407426#article_supplementary_material]*

1.1.1 Treatment Arms in the REMoxTB Study

The control arm in REMoxTB consisted of six month's treatment with standard TB therapy. Ethambutol (E), isoniazid (H), rifampicin (R), and pyrazinamide (Z) were prescribed together for eight weeks as an intensive phase, and then isoniazid and rifampicin only for a total of 26 weeks' treatment (2EHRZ/4HR). The "isoniazid arm" replaced ethambutol with moxifloxacin (M) in the intensive phase of treatment followed by a continuation phase of moxifloxacin, isoniazid, and rifampicin for a total of 17 weeks (2MHRZ/2MHR). The "ethambutol arm" replaced isoniazid with moxifloxacin for

both the intensive phase and continuation phase of treatment, and the total duration was 17 weeks of therapy (2EMRZ/2MR).

Those patients who were found to be eligible during the screening process were randomised to receive either the control, isoniazid, or ethambutol arm. All staff at the local sites and centrally were blinded to patient treatment allocations. Placebo drugs were used to replace moxifloxacin, ethambutol, isoniazid, and rifampicin if these were not contained in the allocated study treatment regimen to maintain blinding. Patients allocated to a 17-week regimen received only placebo drugs from week 18 to week 26 as part of this (see Figure 1-1). Block randomisation according to baseline weight was used to ensure equal numbers of patients across the study arms in each weight category at each of the sites.

All regimens involved single daily dosing of trial medication, including placebo medication, and study drug dosing was supervised. The different sites were permitted to use a method of supervision that was most suitable for them, and included directly observed therapy by site staff, supervision by a community engagement worker or family member, or supervision by another patient nominated treatment supervisor. Medication adherence was recorded from data provided by the patients on questioning by site staff, and drug packaging was inspected for remaining medication. The doses were calculated based on categorical weight bands and these are detailed in Table 1-1.

Patients taking 26-week standard TB therapy in the trial were considered to have taken adequate treatment if they had taken a total of at least 42 doses of intensive phase treatment within 70 days of starting treatment and at least 84 doses of continuation phase treatment within 168 days of completing the intensive phase. Additionally, the patient could not have missed more than 42 doses of medication overall in the trial.

For patients receiving the 17-week experimental treatment in the study, adequate treatment was considered to be at least 42 doses of intensive phase treatment within 70 days of starting treatment and at least 42 doses of continuation phase treatment within 84 days of completing the intensive phase. These patients must not have missed no more than 28 doses overall as well.

Drug Name	Weight Banding	Drug Dose
Ethambutol	<40kg	15mg/kg daily rounded to nearest 100 mg
	40kg to ≤55kg	800mg daily
	>55kg to ≤75kg	1200mg daily
	>75kg	1600mg daily
Isoniazid	All weight ranges	300mg daily
Rifampicin	<45kg	450mg daily
	45kg or higher	600mg daily
Pyrazinamide	<40kg	25 mg/kg rounded to nearest 500mg (if <40 kg 1000mg used instead of 500mg)
	45kg to ≤55kg	1000 mg daily
	>55kg to ≤75kg	1500 mg daily
	>75kg	2000 mg daily
Moxifloxacin	All weight ranges	400mg daily

Table 1-1. *TB medication dosing in the REMoxTB Study based on patient weight.*

1.2 Randomised Patients in the REMoxTB Study

The REMoxTB study aimed to investigate the efficacy and safety of the two experimental, moxifloxacin-containing treatment arms in patients with sputum smear-positive, drug-sensitive pulmonary TB. Patients presenting to the local healthcare services with smear-positive pulmonary TB were eligible for screening by the sites in

the study and subsequently randomised based on the study inclusion and exclusion criteria.

Signed written consent or witnessed oral consent in the case of insufficient literacy was taken before any study-related procedures were undertaken. All randomised patients agreed to any data and samples collected as part of the trial being used in further studies to improve the diagnosis and treatment of tuberculosis, as stated on the informed consent form for the study. All the research activities and data collection for the study was compliant with the Helsinki Declaration and the principles of Good Clinical Practice.

Patients aged over 18 years old who had never been treated for TB were eligible for participation in the study. Two smear-positive sputum samples positive for tubercle bacilli were also required for inclusion in the study, and at least one of these samples must have been confirmed by the REMoxTB study laboratory at the local laboratory. All patients were tested for HIV at screening, and HIV-positive patients were eligible to take part if their CD4+ cell count was greater than or equal to 250 cells/mm^3 and they were not taking antiretroviral medication. A full medical history was taken with clinical examination to ensure there were no pre-existing conditions or contra-indications to trial medications that would exclude the patient. Pre-menopausal women not using an intra-uterine device or surgically sterilised were required to use barrier contraception for the duration of the trial. Patients found to be ineligible during screening were referred to the National Treatment Program for further management of their TB. The inclusion criteria for the trial were:

- Signed written consent or witnessed oral consent in the case of illiteracy, before undertaking a trial related activity

- Two sputum specimens positive for tubercle bacilli on direct smear microscopy of which one confirmed by the REMoxTB study laboratory at the local laboratory
- No history of previous anti-tuberculosis chemotherapy
- Aged 18 years and over
- A firm home address that is readily accessible for visiting and willingness to inform the study team of any change of address and follow-up period
- Agreement to participate in the study and to give a sample of blood for HIV testing
- Negative pregnancy test (women of childbearing potential)
- Pre-menopausal women must be using a barrier form of contraception or be surgically sterilised or have an IUCD in place
- Laboratory parameters performed at least 14 days prior to enrolment:
    - Serum AST and ALT less than 3xULN
    - Serum total bilirubin level less than 2.5xULN
    - Creatinine clearance (CrCl) level greater than 30 mL/min
    - Haemoglobin level of at least 7.0 g/dL
    - Platelet count of at least $50x10^9$ cells/L
    - Serum potassium greater than 3.5 mmol/L

The trial exclusion criteria were:

- Patients unable to take oral medication
- Previously enrolled in this study
- Receiving any investigational drug in the past 3 months or an antibiotic active against M. tuberculosis
- Pregnancy or breast-feeding

- Any condition that may prove fatal during the first two months of the study period
- Severe tuberculosis with high risk of a poor outcome (e.g., meningitis)
- A pre-existing condition likely to prejudice the response to, or assessment of treatment, a condition likely to lead to uncooperative behaviour
- A contraindication to any medications in the study regimens
- A congenital or sporadic cardiac syndrome or taking medications that could result in QTc prolongation
- Patients already receiving anti-retroviral therapy
- Weight less than 35kg
- HIV infection with CD4 count less than 250 cells/μL
- End stage liver failure (class Child-Pugh C)
- Patients whose initial isolate was shown to be multiple drug resistant or mono-resistant to rifampicin, or any fluoroquinolone

1.3 The Patient Assessment Schedule for REMoxTB

The visit schedule in relation to all three of the treatment arms in REMoxTB can be seen in Figure 1-1. The total period for both treatment and follow up in the trial was 18 months from the point of randomisation. Patients were assessed weekly for the first 8 weeks of treatment, monthly until week 26 following the initiation of treatment, and then 3-monthly for a further 12 months. Visit windows were ±3 days for weekly visits, ±14 days for monthly visits, and ±6 weeks for 3 monthly visits. Patients were also reviewed with indicated investigations performed at unscheduled visits, at any time when thought to be necessary by the treating physician. The patient assessment schedule with associated procedures and investigations is shown in Figure 1-2.

1.4 Laboratory Methods in the REMoxTB Study

Standardised laboratory techniques were employed across all the sites in REMoxTB following training by the trial staff. Regular laboratory monitoring visits were carried out through the trial to ensure that laboratory performance was of an acceptable standard and to address any issues or problems. Some site-specific variations in practice were agreed to improve operational aspects of the laboratory's activity while minimising the chance of quality being compromised. Full details of the laboratory procedures in the REMoxTB study can be found in the Laboratory Manual for the trial, and this is available from the UCL Centre for Clinical Microbiology resources website (www.ucl.ac.uk/infection-immunity/centre-clinical-microbiology/resources).

Summary Chart of Visits and Procedures																		
		Active Treatment Phase													Follow-Up Phase			
Activity	**Screening**	**Base line**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 12**	**Week 17**	**Week 22**	**Week 26**	**Mnth 9**	**Mnth 12**	**Mnth 15**	**Mnth 18**
Inclusion/ Exclusion	X																	
Physical examination and vital signs		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Visual Tests		X								X								
Urinalysis	X																	
Sputum smear and culture[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver function[b]	X			X		X				X	X	X						
PT, PTT[b]	X			X		X				X	X	X						
Full blood picture[b]	X			X						X	X	X						
Urea and electrolytes[b]	X			X						X	X	X						
Pregnancy test[c]	X													X				
Susceptibility testing[d]		X																
Mycobacterial typing[e]		X																
Adverse event screening			X	X	X	X	X	X	X	X	X	X	X	X	X			
HIV testing	X																	
CD4 Count[f]	X																	
Chest X-ray		X																

a. One sputum sample will be collected up to and including week 26. Two sputum samples will be collected at month 9, 12, 15 and 18
b. Additional samples may be processed as clinically indicated by the managing clinic staff
c. If applicable
d. Additional susceptibility testing must be performed on any treatment failure or suspected relapse strain
e. Mycobacterial typing should be performed on all initial strains and any treatment failure or suspected relapse strain
f. CD4 count will only be performed on patients who are HIV positive

Figure 1-2. *Patient assessment schedule as detailed in REMoxTB Study Protocol version 1.3. [Available from www.nejm.org]*

1.5 Data Collection in REMoxTB

Data relating to efficacy and safety was collected locally at sites using the paper Clinical Research Forms (CRFs) presented in the Appendix. The data fields on the paper forms were then transcribed into an online database that was managed centrally in the MRC CTU at UCL. During the course of the trial, data entry was monitored and reviewed regularly with data queries generated for the sites to address missing data or inconsistencies identifies by the data scientists. Monitoring visits included checking CRFs against the source documentation where available and data entry against the CRF entry.

At the end of the trial, the database underwent a cleaning process involving both data scientists and statisticians. The data sets were ordered according to the principles of tidy data (Wickham, 2014) and stored at the MRC CTU at UCL.

1.6 Study Outcomes in REMoxTB

Patients in the REMoxTB study were considered to be one of unfavourable, favourable, or not assessable at the end of their follow up period. In this thesis, unless otherwise stated, these outcome measures were not used and instead microbiologically-defined outcome measures were employed. The trial outcomes were defined as follows:

- Unfavourable outcome
    - Patients who required an extension of their treatment beyond that permitted by the protocol, a re-start or a change of treatment for any reason except re-infection or pregnancy.

    - Patients who had a positive culture when last seen (with the exception of patients found to have been re-infected) whether confirmed by a second sample or not.
    - Patients dying from any cause during treatment.
    - Patients failing to complete treatment and not assessable at 18 months.
- Favourable outcome
    - Patients with a negative culture result at 18 months who had not already been classified as having an unfavourable outcome.
- Not assessable
    - Patients who, having completed active treatment, default from follow-up and their last culture was negative.
    - Women who became pregnant during the treatment phase and stopped their allocated treatment, unless their last culture was positive in which case they were classified as having an unfavourable outcome.
    - Patients who died during the follow-up phase with no evidence of failure or relapse of their TB.
    - Patients re-infected with a new strain different from that with which they were originally infected.

A per-protocol (PP) and modified intention-to-treat (mITT) analysis was carried out for the original publication. The exclusion criteria for the mITT analysis were:

1. Patients with MDR disease documented from samples taken at enrolment or week 1 (late exclusions from the study).
2. Patients without culture confirmation of tuberculosis at enrolment or weeks 1 or 2 around enrolment, from screening to week 2 (late exclusions from the study).

3. Patients withdrawn from treatment because of a protocol violation at enrolment (late exclusions from the study, based on data collected prior to randomisation).
4. Patients who, having completed the treatment phase at Month 6, are lost to follow-up or withdrawn from the study, their last status being culture negative and their last positive culture result ("isolated positive culture") followed by at least two negative culture results (without an intervening positive culture).
5. Women who become pregnant during the 6-month treatment phase and stop their allocated treatment.
6. Patients who died during the treatment phase from violent or accidental cause (e.g. road traffic accident). N.B.: This does not include death from suicide, which will be considered an unfavourable outcome.
7. Patients who died during the follow-up phase with no evidence of failure or relapse of their TB, their last status being culture negative and their last positive culture result ("isolated positive culture") followed by at least two negative culture results, and who have not already been classified as unfavourable.
8. Patients who, after being classified as having culture negative status, are re-infected with a new strain different from that with which they were originally infected. "Reinfection" will be defined specifically as a patient infected with a strain that is different from the initial strain as defined by MIRU and IS6110 typing. Assessments of relapse vs. reinfection were made before database lock and unblinding.
9. Patients who are able to produce sputum at 18 months, but whose 18-month visit sputum samples are all (L-J and MGIT) contaminated or missing, who cannot be brought back for repeat cultures, provided they have not already been classified as unfavourable and provided their last positive culture was

followed by at least two negative cultures. N.B.: This does not apply to patients who are unable to produce sputum at 18 months, or to patients who are able to be brought back subsequently and produce negative cultures.

Further exclusion criteria applied to the PP analysis:

1. Patients not meeting the definition of having received an adequate amount of their allocated study regimen, provided they have not already been classified as having an unfavourable outcome.
2. Patients lost to follow-up or withdrawn before the Month 6 visit, unless they have already been classified as having an unfavourable outcome.
3. Patients whose treatment was modified or extended for reasons (e.g. an adverse drug reaction or pregnancy) other than an unfavourable therapeutic response to treatment, unless they have already been classified as having an unfavourable outcome.
4. Patients who are classified as “major protocol violations”, unless they have already been classified as having an unfavourable outcome on the basis of data obtained prior to the protocol violation.

1.7 Withdrawal from the Study and Follow-up

The Sponsor and the site Investigators had the right to withdraw a patient from treatment in the study in the event of a serious or severe adverse event. Patients who were withdrawn from study treatment were to be followed up according to the trial protocol visit schedule for assessment and investigations including sputum sampling, unless the patient also withdrew their consent to follow up. If further treatment for tuberculosis was required, patients were referred to the National Treatment Program by the site staff.

2 Statistical Methods

The REMoxTB study database was available through the Medical Research Council Clinical Trials Unit at University College London (MRC CTU at UCL). All data manipulation and statistical analysis was performed using Stata version 14 (StataCorp, Texas, USA), and statistical significance was set at 5% throughout the thesis.

2.1 Data Distribution

Continuous variables were assessed for their distribution before any statistical tests were performed. The data was visualised as a histogram to determine if the spread was unimodal or multi-modal and to detect skewness in the data. Inverse normal plotting was used to assess whether the data was normally distributed. If all variables been investigated were thought to be approximately normally distributed then parametric testing was used, but if any of the variables under investigation were thought to be not normally distributed then a non-parametric alternative was used instead. Non-parametric methods were also used if one or more of the variables being tested had extreme outliers that could influence the mean.

2.2 Tests for Variable Association or Difference

The Mann-Whitney U test (Mann and Whitney, 1947) was used to test for significant differences in continuous variables between the three treatment arms in the REMoxTB study. This test does require an assumption of normality to produce meaningful results, and is also robust when used to test differences between normally distributed variables. The Chi squared test (Pearson, 1900) was employed to test for significant differences in the proportions of patients seen in categorical variables. This test assumes the predicted values in each square of a frequency table, showing the

numbers belonging to each category, will be greater than 5 and if this was not the case then Fisher's exact test (Fisher, 1922) was used instead. The Kruskal-Wallis test (Kruskal and Wallis, 1952) was used in this thesis as a non-parametric alternative to an analysis of variance. This test was used in cases when a continuous variable was compared across the three study treatment arms to detect statistically significant differences, as opposed to the Mann-Whitney test which was used for non-parametric pairwise comparisons in this thesis.

In cases where an association was investigated between a binary outcome variable and one or more exposure variables, logistic regression was used to test for significant associations. The assumptions that had to be met for this test were that the observations were independent of each other, that there was little or no multicollinearity among the exposure variables, a linear relationship between the exposure variables and the log odds, and lastly that there was a minimum of 10 patients with the least frequent outcome for each independent variable in the regression model. The Hosmer and Lemeshow test (Hosmer and Lemeshow, 1980) was used as a regression diagnostic for the models used in the thesis.

Zellner's seemingly unrelated regression (Zellner, 1962) was used as a multivariate regression test. This multivariate regression uses simultaneous generalised linear regression for a number of outcomes of interest in relation to one or more exposure variables, but there is an assumption that the covariance for the error terms between the individual linear regression models is non-zero. Therefore, a degree of relatedness between the outcome variables is assumed and the final regression coefficients for each outcome variable can be interpreted as the association with the exposure variable of interest, with an accounting for any relatedness between the outcomes. Hence, the term "seemingly unrelated". This is a feasible least squares technique, and

there is a lot of overlap with ordinary least squares calculations. The initial step is to use ordinary least squares regression for each of the outcome variables against the exposure variables as univariable regressions, and the residuals are used to create a matrix. Following on from this, a generalised least squares regression is carried out using this residual matrix as a variance matrix for each of the outcome variables again in univariable regressions against the exposure of interest. As a result, the error estimator for the equations uses shared variance from across the univariable linear equations and allows for interpretation of each outcome in relationship to the other outcomes. When this test was utilised, the assumptions considered were the same as for ordinary least squares: that the linear regression model was effectively linear in its parameters, there was random sampling of the observations, the conditional mean would be zero, and that there was no multicollinearity.

2.3 Time to Event Analyses

The **`stset`** command in Stata was used for data relating to time-to-event analyses. This provides the software with the information it needs to label events in relation to the start of treatment in the study, and creates further variables relating to the timing of any events of interest for use in further tests and graphs. Kaplan-Meier curves (Kaplan and Meier, 1958) were constructed using the **`sts graph`** command with risk tables added as an argument to the command.

The logrank test (Mantel, 1966; Peto and Peto, 1972) was used to test for differences between study treatment arms. The null hypothesis that there is no difference between the two groups for the probability of the event of interest at any time point, and a p value is calculated to that effect. The test involves calculating the observed number of events in each group at each time point when an event occurs, and the number that would be expected if there was no difference between the groups.

Cox regression (Cox, 1972; Breslow, 1975) was used to generate hazard ratios for the time to the event of interest based on selected exposure variables. This test operates under the proportional hazards assumption, meaning the effects of exposure variables upon the time to the event are constant over time and proportional across the groups being tested (ie the ratio of the hazards for any two individuals is constant over time). The proportional hazards assumption was checked using the **`stphplot`** command to generate log-log plots (ensuring they do not cross) and **`estat phtest`** to test the assumption using Schoenfeld residuals (Schoenfeld, 1981) after the Cox regression model was fitted.

2.4 User-written Packages

User-written packages were utilised in the thesis. However, a package must have been published in a peer-reviewed journal specialising in statistical analysis or software programming (such as the Stata Journal). Where user-written packages have been used as part of an analysis, a reference to the publication has been provided and the package has been explicitly named with a description of its application.

Chapter Three: Adverse Events and Toxicity Related to Tuberculosis Treatment

1 General Introduction

The toxicity associated with standard tuberculosis (TB) therapy is poorly characterised in the existing literature. Isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E) have been used in combination to treat TB for several decades and there are recognised side effects from these drugs when they are used to treat TB: including peripheral neuropathy (Zilber *et al.*, 1963; Schaberg, Rebhan and Lode, 1996), gastrointestinal upset (Yee *et al.*, 2003), optic neuropathy (Marra *et al.*, 2007), and liver injury (Ormerod *et al.*, 1998; Saukkonen *et al.*, 2006). However, the interpretation of the existing publications is impaired by varying definitions for reporting toxicity (Devarbhavi, 2011; Saukkonen, Powell and Jereb, 2012), and the retrospective or observational nature of the majority of studies. There are few prospective studies designed to investigate efficacy and safety, largely due to the lack of new drugs until recently (Pai *et al.*, 2016). This can lead to clinical practice being predominantly guided by personal experience and anecdotal evidence, which results in variable approaches to the management of drug side effects and monitoring for toxicity.

The patient characteristics associated with toxicity are also variably reported. Female gender, human immunodeficiency virus (HIV) infection, alcohol dependence, and

some ethnic groups have been identified as being at higher risk for experiencing adverse events (AEs) on TB treatment (Yee *et al.*, 2003; Marra *et al.*, 2007; Pepper *et al.*, 2010; Lorent *et al.*, 2011; McIlleron *et al.*, 2015; Sadiq *et al.*, 2015; Vasakova, 2015). The lack of a consistent approach to defining and reporting across these studies results in variable risks being reported. As an example, the rates of ethambutol-associated visual impairment range from 0.02% to greater than 35% in the literature (Fraunfelder, Sadun and Wood, 2006; Menon *et al.*, 2009; Chen *et al.*, 2012; Ezer *et al.*, 2013), despite the importance of this complication and the relative simplicity of testing for its presence with little training required.

Toxicity related to treatment has been associated with poorer outcomes in TB to different extents (Lorent *et al.*, 2011; Wohlleben *et al.*, 2017). Side effects such as hepatotoxicity have been linked with treatment interruptions (Sharma *et al.*, 2010), and increased morbidity and mortality (Saukkonen *et al.*, 2006; Shang *et al.*, 2011). Additionally, less severe drug toxicities such as gastrointestinal upset have been reported as factors related to patient non-compliance and loss to follow up in TB clinics (Chida *et al.*, 2015; Gugssa Boru, Shimels and Bilal, 2016).

In this chapter, the aim was to investigate the pattern of toxicity associated with standard TB therapy using a large prospectively collected database. Specifically, to answer the following questions:

- Who is affected by toxicity?
- When does it happen and what form does it most commonly take?
- What is the impact of experiencing toxicity on treatment outcomes?
- How does the toxicity associated with standard TB therapy compare to the toxicity seen in the experimental arms used in REMoxTB?

2 Baseline Characteristics for Patients taking Standard TB Therapy

The TB physician needs to be able to identify those patients that are at highest risk for experiencing clinically significant toxicity. This has implications on how monitoring programs will be set up to ensure that the most vulnerable patient groups are kept under close review and any toxicity that could potentially have a negative impact on their treatment is acted upon at an early stage. There are also considerations for clinical trials where eligible patients need to be identified and patients have to give informed consent before participation, and if a patient belongs to a group at higher risk of adverse events they should be made aware of this fact.

This section uses the data relating to patient characteristics to investigate for any significant associations between patient characteristics at the beginning of treatment and reported adverse events while taking standard TB therapy.

2.1 Methods

2.1.1 Adverse Event Definitions

In REMoxTB adverse events (AEs) were recorded for all randomised patients during both the treatment and follow up phases of the trial. AEs were defined as any untoward medical occurrence in a patient who has been administered trial medication, with or without a causal relationship to the drugs. All AEs were graded in terms of clinical severity by the site doctor on a scale from grade 1 (least severe) through to grade 4 (most severe). The grading system worked to specific criteria depending on the nature of the individual event, for example test results could be graded based on numerical cut-offs while symptoms were graded based on the reported and observed severity, and adhered to the Division of AIDS of the National Institute of Allergy and Infectious

Diseases (DAIDS) criteria. To act as an illustration, Table 2-1 demonstrates a sample of severity grading criteria using events that are both clinically and numerically graded taken from the DAIDS Tables for Grading the Severity of Adult and Paediatric Adverse Events Version 2.0 (November 2014). The DAIDS guidelines also state that any AE that ends in death should be reported as “grade 5”, but in REMoxTB these events were identified when reported as Serious Adverse Events (SAEs). For the purposes of this chapter, events that were grade 3 or 4 in severity were considered “clinically significant” based on the assumption that these are the events that would generally be acted upon in routine clinical practice. AEs were considered “serious” if they fulfilled any of the standard criteria, regardless of the severity grading. These criteria were:

- Death from any cause
- Hospitalisation for any reason
- Events that were considered life-threatening by the clinician
- Congenital abnormalities
- Persistent or significant disability
- Medical significance, as judged by the site doctor

The local clinicians made an assessment for relatedness to the trial medication for each AE and this would be based on the timing of the event, known side effects of the treatment, and the effects of re-starting medication after a treatment pause. Those AEs that were classified as “possibly”, “probably” or “definitely” related to trial medication were classed as “related” for this chapter’s analyses. Any AEs that were considered “not related” or “unlikely related” by the site doctors were classed as “not related” for this chapter.

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Clinical adverse event **NOT** identified elsewhere in the grading table	Mild symptoms causing no or minimal interference with usual social & functional activities with intervention not indicated	Moderate symptoms causing greater than minimal interference with usual social & functional activities with intervention indicated	Severe symptoms causing inability to perform usual social & functional activities with intervention or hospitalization indicated	Potentially life-threatening symptoms causing inability to perform basic self-care functions with intervention indicated to prevent permanent impairment, persistent disability, or death
Prolonged QTc Interval	0.45 to 0.47 seconds	> 0.47 to 0.50 seconds	> 0.50 seconds OR ≥ 0.06 seconds above baseline	Life-threatening consequences (e.g., Torsade de pointes, other associated serious ventricular dysrhythmia)
Vomiting	Transient or intermittent AND No or minimal interference with oral intake	Frequent episodes with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Creatinine, High	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	>1.8 to<3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Vertigo	Vertigo causing no or minimal interference with usual social & functional activities	Vertigo causing greater than minimal interference with usual social & functional activities	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions

Table 2-1. Sample entries from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0 (Nov 2014). [Obtained from https://rsc.tech-res.com/docs/default-source/safety/daids_ae_grading_table_v2_nov2014.pdf]

The AEs in REMoxTB were coded using the MedDRA coding system and allocated a System Organ Class (SOC) and Preferred Term (PT) based on the nature of the event. Having reviewed the SOCs and PTs for a sample of the AEs, it was decided that the

most clinically relevant approach to analysing this data was to predominantly use the SOC as this would give the reader a better grasp of the overall trends in AEs. However, as a result of the coding process there were many AEs grouped under the "Investigations" SOC because the PT related to the test result rather than the pathology underlying it (e.g. "low haemoglobin" as opposed to "anaemia") and this meant that a lot of meaningful information would be lost if the SOCs were used in their natural state. Therefore, the majority of the AEs classed as "Investigations" were manually recoded into SOCs that better reflected the nature of the event (e.g. "raised ALT" was reallocated to "Hepatobiliary Disorders").

2.1.2 Baseline Characteristics and Adverse Events

Baseline characteristics (e.g. sex, weight, age, ethnicity) were available for all the patients allocated to the standard therapy arm in REMoxTB. Patients were grouped according to whether they experienced no clinically significant AEs (grade 3 or 4 severity), ≥1 clinically significant AE, or ≥1 clinically significant AE assessed as related to treatment. The proportions of patients belonging to each group was tabulated to facilitate visual comparison between the group with no events and the group of patients with ≥1 (total or related-only) AEs according to individual baseline characteristics. Age and weight were both converted from continuous to categorical variables for the table and for use in the regression analyses.

Univariable logistic regression was used to test the relationship between the baseline characteristics and the binary outcome variable of ≥1 clinically significant AE. Baseline characteristics were used as independent variables and ≥1 grade 3 or 4 AE was used as the binary dependent variable to generate odds ratios. Separate univariable logistic regression analyses were carried out using the total number of grade 3 or 4 AEs, and also in regression analyses using only the grade 3 or 4 AEs judged to be related to

trial medication by the site doctor. Ethnicity was considered a factor variable with "black" used as the baseline for the variable due to the largest number of patients belonging to this group. Time to Mycobacterial Growth Indicator Tube (MGIT) positivity, as a marker of bacillary load, was included in the regression analysis as a continuous variable.

Characteristics with a p value of <0.10 in the manual univariable regression were chosen for inclusion in a random-effects multivariable logistic regression model. Separate regression analyses were carried out using the total grade 3 or 4 AEs, and those events thought to be related only. Age, gender, and baseline weight were included in the multivariable models looking for association with total and related grade 3 or 4 AEs regardless of the p value. This was based on the clinical judgement that these patient characteristics should be included to weight the model regardless of statistical significance, as they would be expected to have an impact on a patient's clinical course. Random-effects multivariable logistic regression was used to test for associations between the selected variables and grade 3or 4 AEs with trial centre used as the panel variable to account for any effect from the individual sites.

2.2 Results

Of the 639 patients taking standard therapy, 128 (20.0%) experienced ≥1 grade 3 or 4 AE during the trial. Table 2-2 describes the baseline characteristics for all patients on the standard therapy arm based on whether the patient experienced ≥1 grade 3 or 4 AE. There were higher proportions of females compared to males (25.5% vs 17.7%), patients with baseline weight ≤45kg compared to >45kg (56.8% vs 43.2%), and HIV-positive versus HIV-negative patients (43.5% vs 18.2%) experiencing ≥1 grade 3 or 4 AE. Cavities on chest X-ray and smoking history did not show higher proportions than

the overall proportion of 20.0% for patients experiencing events. The median time-to-positivity (TTP) in liquid culture was 111 hours and 116 hours among those patients who did and did not experience ≥1 grade 3 or 4 AE, respectively.

		Zero Grade 3/4 AEs	≥1 Grade 3/4 AEs	Total
No of subjects (% total)		511 (80.0%)	128 (20.0%)	639
Gender (%)				
	Male	368 (82.3%)	79 (17.7%)	447
	Female	143 (74.5%)	49 (25.5%)	192
Age (%)				
	<25 yrs	150 (80.2%)	37 (19.8%)	187
	25-35 yrs	155 (84.2%)	29 (15.8%)	184
	>35 yrs	206 (76.9%)	62 (23.1%)	268
Baseline Weight (%)				
	<40 kg	45 (71.4%)	18 (28.6%)	63
	40-45 kg	74 (71.8%)	29 (28.2%)	103
	>45-55 kg	214 (84.3%)	40 (19.7%)	254
	>55-75 kg	163 (80.3%)	40 (19.7%)	203
	>75 kg	15 (93.8%)	1 (6.2%)	16
Ethnicity (%)				
	Black	237 (80.3%)	58 (19.7%)	295
	Asian	147 (75.8%)	47 (24.2%)	194
	Mixed Race	119(85.0%)	21 (15.0%)	140
	Other	8 (80.0%)	2 (20.0%)	10
Smoking History (%)				
	Never	235 (78.9%)	63 (21.1%)	298
	Ex-smoker	126 (81.3%)	29 (18.7%)	155
	Current	150 (80.7%)	36 (19.3%)	186
HIV Status (%)				
	Positive	26 (56.5%)	20 (43.5%)	46
	Negative	485 (81.8%)	108 (18.2%)	593
Cavities on CXR*				
	Yes (%)	366 (80.3%)	90 (19.7%)	456
	No (%)	96 (80.7%)	23 (19.3%)	119
MGIT Median TTP in hours (IQR)		116 (89 – 154)	111 (86 – 163)	114 (88-156)

Table 2-2. Baseline characteristics of patients allocated to standard therapy grouped according to the number of grade 3 or 4 AEs experienced during the course of the trial. Row totals provided for patients based on characteristic and row percentages given in each cell. Note that population totals vary depending on whether timing of event is considered, as there was no start date recorded for some AEs *Note some X-rays were either unobtainable or unsuitable for reporting

Table 2-3 presents the output from univariable and random-effects multivariable logistic regression of baseline characteristics against a binary outcome of ≥1 grade 3 or 4 AE. HIV infection (adjOR 3.43, 95% CI 1.82 – 6.49) was the only baseline characteristic found to be significantly related to grade 3 or 4 AEs in the multivariable

model. While female sex (OR 1.60, 95% CI 1.06 – 2.39) and baseline weight (OR 0.79, 95% CI 0.65 – 0.97) were significantly associated with experiencing an event in the univariable analysis, these characteristics failed to maintain their significance in the multivariable model. Age, ethnicity, smoking history and measures of bacillary burden (cavitation on chest X-ray and MGIT time-to-positivity) were not significantly associated with increased odds of experiencing ≥1 grade 3 or 4 AE.

Baseline Characteristic	OR	95% CI	P value	adjOR	95% CI	P value
Female Gender	1.60	1.06 – 2.39	0.02	1.36	0.87 – 2.13	0.18
Age*	1.13	0.90 – 1.43	0.30	1.15	0.90 – 1.47	0.25
Baseline Weight*	0.79	0.65 – 0.97	0.02	0.79	0.63 – 1.03	0.08
Ethnicity						
Black	BASELINE	---	---	---	---	---
Asian	1.31	0.84 – 2.02	0.23	---	---	---
Mix Race	0.72	0.42 – 1.24	0.24	---	---	---
Smoking Hist.						
Never	BASELINE	---	---	---	---	---
Ex-smoker	0.86	0.53 – 1.40	0.54	---	---	---
Current	0.90	0.57 – 1.42	0.64	---	---	---
HIV Positive	3.45	1.86 – 6.42	<0.001	3.43	1.82 – 6.49	<0.001
Cavities on CXR	1.03	0.62 – 1.71	0.92	---	---	---
Baseline TTP	1.00	1.00 – 1.00	0.46	---	---	---

Table 2-3. Output from univariable and random-effects multivariable logistic regression with ≥1 grade 3 or 4 AE as outcome. OR= Odds ratio, adjOR= Adjusted OR, 95% CI=95% confidence intervals *Age and baseline weight were categorised as presented in Table 3 of this chapter

There were 57 of the 639 (8.9%) standard therapy arm patients who reported ≥1 grade 3 or 4 AE thought to be related to standard TB therapy. Table 2-4 shows the baseline characteristics for all the patients taking standard TB therapy, with numbers grouped according to the number of related grade 3 or 4 AEs experienced. HIV positive patients (23.9%), patients with a baseline weight 45kg or less (13.3%), and female patients

(13.5%) had higher proportions with ≥1 related grade 3 or 4 AE compare to the overall proportion of 8.9%. While patients who were labelled “Other” ethnicity had the highest proportion of the different ethnic groups at 20%, this was based on a total number of ten patients.

		Zero Related Grade 3/4 AEs	≥1 Related Grade 3/4 AEs	Total
No of subjects (% total)		582 (91.1%)	57 (8.9%)	639
Gender (%)				
	Male	416 (93.1%)	31 (6.9%)	447
	Female	166 (86.5%)	26 (13.5%)	192
Age (%)				
	<25 yrs	176 (94.1%)	11 (5.9%)	187
	25-35 yrs	164 (89.1%)	20 (10.9%)	184
	>35 yrs	242 (90.3%)	26 (9.7%)	268
Baseline Weight (%)				
	<40 kg	55 (87.3%)	8 (12.7%)	63
	40-45 kg	89 (86.4%)	14 (13.6%)	103
	>45-55 kg	241 (94.9%)	13 (5.1%)	254
	>55-75 kg	181 (89.2%)	22 (10.8%)	203
	>75 kg	16 (100%)	0 (0.0%)	16
Ethnicity(%)				
	Black	269 (91.2%)	26 (8.8%)	295
	Asian	174 (89.7%)	20 (10.3%)	194
	Mixed Race	131 (93.6%)	9 (6.4%)	140
	Other	8 (80.0%)	2 (20.0%)	10
Smoking History (%)				
	Never	271 (90.9%)	27 (9.1%)	298
	Ex-smoker	139 (89.7%)	16 (10.3%)	155
	Current	172 (92.5%)	57 (7.5%)	186
HIV Status (%)				
	Positive	35 (76.1%)	11 (23.9%)	46
	Negative	547 (92.2%)	46(7.8%)	593
Cavities on CXR				
	Yes (%)	424 (93.0%)	32 (7.0%)	456
	No (%)	106 (89.1%)	13 (10.9%)	119
MGIT Median TTP (IQR)		114 (88 – 155)	118 (95 – 173)	114 (88-156)

Table 2-4. Baseline characteristics of patients allocated to standard therapy grouped according to the number of related grade 3 or 4 AEs experienced during the course of the trial. Row totals provided for patients based on characteristic and row percentages given in each cell. Note that population totals vary depending on whether timing of event is considered, as there was no start date recorded for some AEs

Female gender (adjOR 1.97, 95% CI 0.91 – 1.83) and HIV-positive status (adjOR 3.33, 95% CI 1.55 – 7.14) were both found to be significantly associated with experiencing

≥1 related grade 3 or 4 AE in the random-effects multivariable regression model shown in Table 2-5. Baseline weight was not found to be significant in either the univariable or multivariable regression. Similar to the total events, none of the other characteristics were found to be significant when analysed as an univariable exposure in logistic regression with the outcome of ≥1 related grade 3 or 4 AE.

Baseline Characteristic		OR	95% CI	P value	adjOR	95% CI	P value
Female Gender		2.10	1.21 – 3.65	0.01	1.96	1.08 – 3.56	0.03
Age*		1.25	0.89 – 1.74	0.20	1.29	0.91 – 1.83	0.16
Baseline Weight*		0.86	0.65 – 1.14	0.30	0.90	0.67 – 1.21	0.49
Ethnicity							
	Black	BASELINE	---	---	---	---	---
	Asian	1.19	0.64 – 2.20	0.58	---	---	---
	Mix Race	0.71	0.32 – 1.56	0.40	---	---	---
Smoking Hist.							
	Never	BASELINE	---	---	---	---	---
	Ex-smoker	1.16	0.60 – 2.22	0.66	---	---	---
	Current	0.82	0.42 – 1.60	0.56	---	---	---
HIV Positive		3.74	1.78 – 7.84	<0.001	3.33	1.55 – 7.14	0.002
Cavities on CXR		0.62	0.31 – 1.21	0.16	---	---	---
Baseline TTP		1.00	1.00 – 1.00	0.05	1.00	1.00 – 1.01	0.06

Table 2-5. Output from univariable and random-effects multivariable logistic regression with ≥1 related grade 3 or 4 AE as outcome. OR= Odds ratio, adjOR= Adjusted OR, 95% CI=95% confidence intervals. *Age and baseline weight were categorised as presented in Table 3 of this chapter

3 Incidence of Adverse Events while taking Standard TB Therapy

Doctors managing patients with TB disease are frequently working in resource-constrained settings and it is critical that these resources are directed towards the patients with the greatest need. It is clear that appropriate allocation of resources is key to ensuring safe monitoring for patients taking TB treatment. An accurate

description of the timings when complications are most likely, and what form these complications take, would assist in the allocation of these limited resources.

The section is focused on the issue of providing evidence for guidance for physicians developing monitoring schedules by elucidating the time periods during treatment where the risk of adverse events is greatest. The focus is on standard therapy and its toxicity profile for this section to allow for greater detail, given that this is the regimen that is currently in widespread use for TB treatment.

3.1 Methods

Grade 3 and 4 AEs were categorised according to the treatment phase in which they occurred. The start date for the event was used to categorise events as occurring in either intensive (0-8 weeks), continuation (9-26 weeks), or follow up (26 weeks to 18 months) phases for patients on the standard therapy arm. The difference in weeks between the first dose of medication and the start date of the event was calculated, and the resulting number used to allocate the AE into a treatment phase. The total number of grade 3 and 4 AEs in each phase was counted separately and the proportions of these events that were considered related were calculated. The number of patients who experienced 0, 1, 2 or ≥3 related grade 3 or 4 AEs in each of the treatment phases was tabulated. The number of patients who experienced ≥1 SAE in each treatment phase was also included in the table.

The **`stset`** command was used to create a survival-time dataset relating to the time to first grade 3 or 4 AE. The start date for each event was converted to the day of treatment by creating a new variable containing the time difference in days between the patient's first dose of drug and the event start date. Any events that occurred up to seven days before the first drug dose were considered to have happened on day

"zero", and events that started earlier than this were excluded. This was to ensure that adverse events occurring close to the time of treatment initiation were included, to attempt to characterise the clinical picture for patients initiating treatment and also to include them as part of the analysis looking at the impact on treatment outcomes. A hazard curve was constructed to illustrate the probability of experiencing a clinically significant episode of drug toxicity at different time points after starting treatment. The **`hazard`** argument was used with the **`sts graph`** command to construct a smoothed hazard function with 95% confidence intervals using the weighted kernel smoothed hazard estimate to obtain an instantaneous rate of occurrence for the grade 3 and 4 AEs.

Deaths from any cause and withdrawals from treatment for any reason were also categorised according to the treatment phase in which they occurred. All deaths in the standard arm had a date of death recorded, and the difference between this date and the patient's first dose of trial drug was used to calculate the time variable. Withdrawals in the REMoxTB database were flagged as either "treatment phase" or "follow up", depending on whether the patient was still taking medication at the time of their withdrawal. This analysis included only withdrawals from the treatment phase for two reasons. First, the majority of withdrawals were in the patient's treatment phase, and secondly my main interest in this thesis was to explore the effects of drug toxicity. As with other events, the treatment phase was determined by calculating the difference between the withdrawal date and the first dose date.

3.2 Results

The majority of grade 3 and 4 AEs occurred in the intensive phase of treatment, as Table 3-1 demonstrates. This was the case regardless of the relatedness assessment by the site doctor, with 135 of 250 (54.0%) grade 3 or 4 AEs and 80 of 113 (70.8%)

related grade 3 or 4 AEs reported in the first eight weeks of treatment. Four grade 3 or 4 AEs occurred in follow up that were assessed as “possibly” related to treatment. These occurred between 31 and 61 weeks after starting treatment and related to poor diabetic control, diabetic ketoacidosis, spontaneous abortion, and anaemia. There was only one report of clinically significant deterioration in visual acuity on the standard arm, assessed as probably related to treatment.

The hazard curve in Figure 3-1 demonstrates the adjusted risk for the occurrence of a related grade 3 or 4 AE in weeks since the first dose of standard TB therapy. The curve is non-linear and illustrates that the time period of highest risk for experiencing one of these events lies within the first two months after starting therapy, before rapidly falling in the intensive phase of treatment.

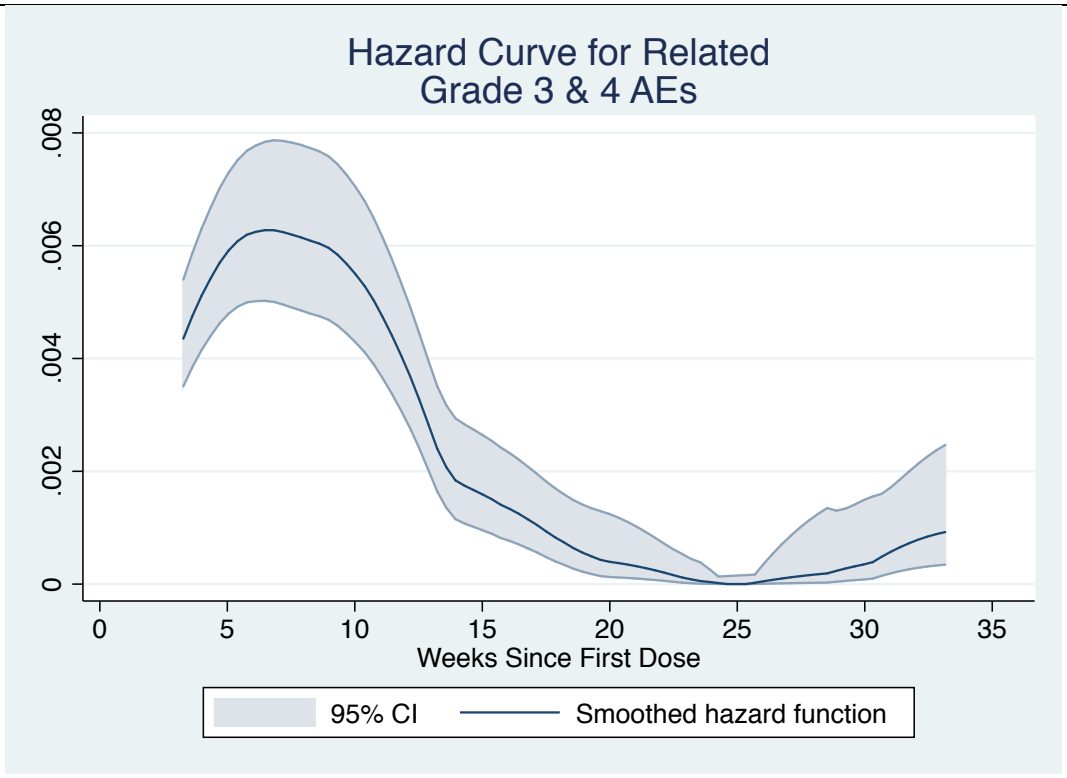

Figure 3-1. Hazard Curve for Grade 3 or 4 Related Adverse Events According to Number of Weeks Since First Dose of Standard Therapy. Hazard function for the occurrence of a grade 3 or 4 related adverse event (with 95% confidence intervals) is plotted on the y axis, with the number of weeks following the first dose of standard tuberculosis therapy on the x axis. The rise in the hazard function after week 25 is accounted for by 2 events reported as “possibly” related to study drug

	Intensive Phase (Weeks 0 – 8) n= 639	Continuation Phase (Weeks 9 – 26) n= 596	Follow-up Phase (Month 7 – 18) n= 569
Total Grade 3 & 4 AEs Reported	135	62	53
Related (% Total in Treat Phase)	80 (59.3%)	29 (46.8%)	4 (7.5%)
No of Grade 3 AEs Reported	100	48	33
Related (%Grade3 in Treat Phase)	50 (50.0%)	23 (57.5%)	3 (9.1%)
No. Grade 4 AEs Reported	35	14	20
Related (%Grade4 in Treat Phase)	30 (85.7%)	6 (42.9%)	1 (5.0%)
System Organ Class of Related Events (% of Total G3 or G4 AEs in Treat Phase)			
Hepatobiliary	25 (18.5%)	13 (21.0%)	0 (0.0%)
Musculoskeletal	15(11.1%)	7 (11.3%)	0 (0.0%)
Metabolism & Nutrition	9 (6.7%)	1 (1.6%)	2 (3.8%)
Blood & Lymphatic	5 (3.7%)	2 (3.2%)	1 (1.9%)
No of Related Grade 3 or 4 AEs per Patient (%n)			
0	592 (92.6%)	581 (97.5%)	566 (99.5%)
1	33 (5.2%)	12 (2.0%)	2 (0.4%)
2	10 (1.6%)	1 (0.2%)	1 (0.2%)
≥3	4 (1.0%)	2 (0.3%)	0 (0.0%)
No of Patients with ≥1 SAE (% n)	32 (5.0%)	18 (3.0%)	20 (3.5%)
No of Patients with ≥1 Related SAE (%n)	17 (2.7%)	3 (0.5%)	2 (0.4%)
Mean No of SAEs per Patient	1.78	1.39	1.60
No of Withdrawals (%n)	38 (5.9%)	26 (4.4%)	1 (0.2%)
No of Deaths (%n)	5 (0.8%)	1 (0.2%)	10 (1.8%)

Table 3-1. Adverse events in standard arm by treatment phase. The number of grade 3 and 4 AEs are shown according to treatment phase along with the most common SOC for related events and the number of grade 3 or 4 AEs experienced by patients in each treatment phase. Serious adverse events are shown in in each treatment phase regardless of severity grading. The treatment phases were not independent and the same patient could appear in more than one phase

The related grade 3 or 4 AEs were most commonly hepatobiliary disorders, musculoskeletal disorders, and related to metabolism or nutrition. Figure 3-2 illustrates

the SOC assignment for all related grade 3 or 4 AEs reported on the standard therapy arm. Hepatobiliary disorders were most commonly “Hepatic enzyme increased” (47.3%), “Gamma-glutamyltransferase increased” (13.2%), and “Alanine aminotransferase increased” (10.5%). Musculoskeletal disorders were predominantly “Arthralgia” (31.8%), “Pain in extremity” (27.3%), or “Joint range of motion decreased” (13.6%). Lastly, metabolism and nutrition disorders most frequently referred to “Hyponatraemia” (33.3%), “Decreased appetite” (25.0%), or “Diabetes/hyperglycaemia/diabetic ketoacidosis” (24.9%). The breakdown of related grade 3 or 4 AEs according to treatment phase can be seen in Table 3-2.

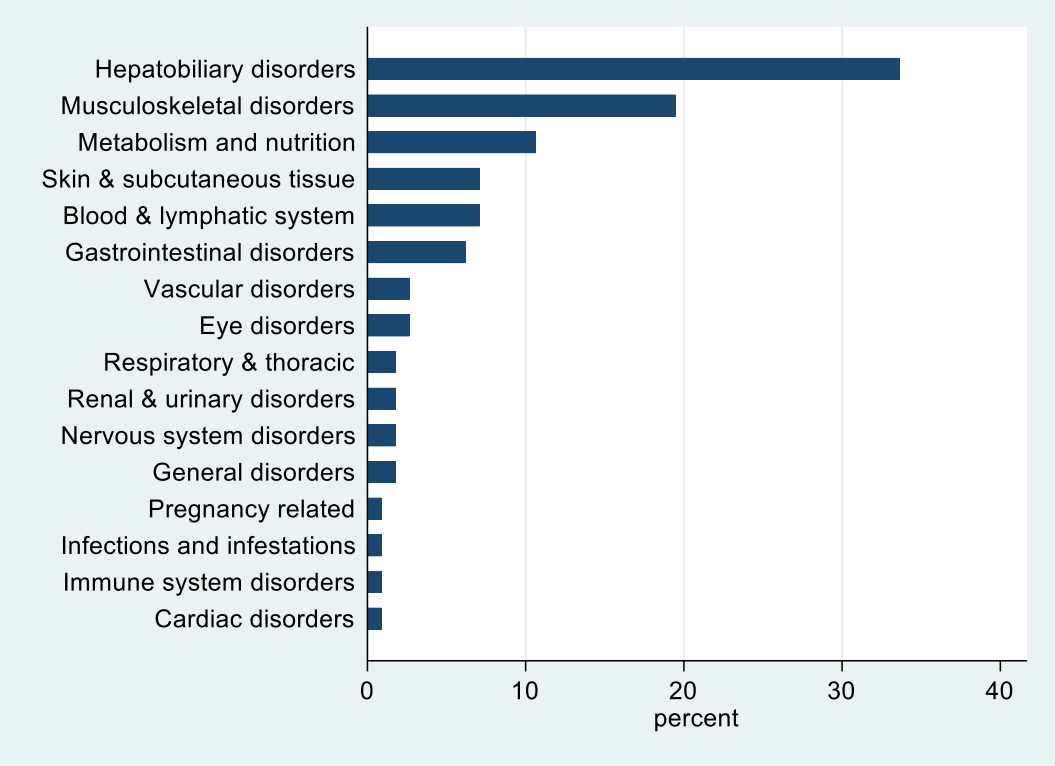

Figure 3-2. *System Organ Class (SOCs) for all related grade 3 or 4 AEs reported for patients taking standard TB therapy. SOCs are arranged by descending frequency and bar chart values correspond to the percentage of the total number of events that fall into the SOC*

The SOCs for the 137 unrelated grade 3 or 4 AEs on standard therapy were most commonly respiratory, thoracic and mediastinal disorders (21.2%), blood and lymphatic disorders (18.3%), or metabolism and nutrition disorders (12.4%).

Hepatobiliary disorders accounted for 8 of 137 events (5.8%). “Haemoptysis” (31.0%), “Dyspnoea” (20.7%), and “Respiratory disorder” (13.8%) were the most commonly reported PTs among the 29 respiratory, thoracic and mediastinal disorders. The majority of the 25 blood and lymphatic disorders were “Haemoglobin decreased” (32.0%), “Anaemia” (20.0%), or “Prothrombin time prolonged” (20.0%). The highest number of reported PTs were “Weight decreased” (41.2%), and “Diabetes/hyperglycaemia” (35.3%) among the 17 Metabolism and Nutrition SOC events.

SYSTEM ORGAN CLASS	TREATMENT PHASE			TOTAL
	INTENSIVE	CONTINUATION	FOLLOW UP	
Hepatobiliary Disorders	25 (65.8%)	13 (34.2%)	0 (0.0%)	38
Musculoskeletal and Connective Tissue	15 (68.2%)	7 (31.8%)	0 (0.0%)	22
Metabolism and Nutrition	9 (75.0%)	1 (8.3%)	2 (16.7%)	12
Blood and Lymphatic System	5 (62.5%)	2 (25.0%)	1 (12.5%)	8
Skin and Subcut. Tissue	6 (75.0%)	2 (25.0%)	0 (0.0%)	8
Gastrointesinal Disorders	6 (85.7%)	1 (14.3%)	0 (0.0%)	7
Eye Disorders	3 (100.0%)	0 (0.0%)	0 (0.0%)	3
Vascular Disorders	3 (100.0%)	0 (0.0%)	0 (0.0%)	3
General Disorders	1 (50.0%)	1 (50.0%)	0 (0.0%)	2
Nervous System Disorders	2 (100.0%)	0 (0.0%)	0 (0.0%)	2

Table 3-2. System Organ Class (SOC) of grade 3 or 4 AEs assessed as related to standard TB therapy according to treatment phase, based on the start date of the event

While the majority of withdrawals from treatment occurred in the intensive phase, the highest number of deaths in the standard treatment arm occurred in follow-up after patients completed their treatment (see Table 3-1). 38 of the 65 (58.5%) withdrawals

from treatment on the standard arm took place during the intensive phase and were most frequently due to “adverse drug reactions/toxicity” (24 of 65, 36.9%) or “withdrawal of consent” (13 of 65, 20.0%). 10 of the 16 (62.5%) deaths among patients taking standard therapy were after the patient had finished taking treatment and none of the 16 deaths were assessed as related to therapy. 8 (50%) of the deaths were reported as due to trauma, suicide, or unknown cause but possibly violent. Only 4 of the 16 (25.0%) deaths were due to TB disease. None of the deaths were assessed as related to standard therapy.

The incidence of related SAEs followed a similar pattern to grade 3 or 4 AEs. Table 3-1 demonstrates that the majority of patients who experienced ≥1 SAE assessed as related to treatment did so in the intensive phase. In comparison, patients who experienced ≥1 SAE (related or unrelated) were more evenly distributed across the phases of treatment.

4 Outcomes from Treatment and the Impact of Toxicity

While the aim in the previous two sections was to identify how best to implement change, in this section the goal was to investigate the association between the incidence of adverse events and outcomes on treatment with standard TB therapy. In order to create impetus for change there must be a demonstration of negative consequences if no action is taken. Policy makers must be convinced to distribute funding and this is achieved by demonstrating the potential for tangible positive impact from change in that area. A well-defined and robust description of the consequences from drug toxicity would help build the case for improving TB services.

This section focusses on the associations between drug toxicity and negative outcomes on treatment with standard TB therapy in the form of treatment failure, withdrawal from the REMoxTB trial, and death from any cause.

4.1 Methods

The reported outcomes for all AEs recorded on standard therapy were collected to investigate how the proportions of events with different outcomes differed according to severity grading and the relatedness assessment. The site doctor provided an outcome for each AE, which would be one of five options: “improved”, “ongoing”, “recovered”, “worsened”, or “death”. AEs were tabulated from the standard therapy arm according to their severity and outcome, with the exception of any events with “death” as an outcome as these events would be grade 4 by necessity. The Chi square test for significance was employed to detect differences in the proportions of events with different outcomes according to severity grading. Following this, the same tabulation and analysis was carried out using the related AEs only. The experimental arms were excluded from the analysis of outcomes as they failed to achieve non-inferiority compared to standard therapy in the original REMoxTB publication.

4.1.1 Defining Treatment Outcomes

Data relating to culture status at eighteen months after randomisation on both Lowenstein-Jensen (LJ) slopes and Mycobacterial Growth Indicator Tube (MGIT) culture were available for the majority of patients randomised into the trial, including most of the patients who were withdrawn from treatment in the trial and referred on to the National Treatment Program. For the purposes of this analysis, patients who were culture negative at eighteen months were labelled as “cured”. If a patient was lost to follow up, or died before eighteen months in the trial, then they were considered cured if they had completed their treatment and had two or more consecutive negative

cultures (at different visits) prior to the date that they were last seen. Patients were considered as not having achieved cure if they were culture positive at eighteen months, were lost to follow up, or died with less than two consecutive negative cultures immediately prior.

To generate more detailed treatment outcome groups the patients on standard therapy were further classified into one of three groups relating to treatment completion in the trial and death from any cause. Within these three categories patients were then labelled as either “cured” or “not cured” based on the definition above. This resulted in six different outcomes:

1. Completed trial treatment and cured
2. Completed trial treatment and not cured
3. Early withdrawal from treatment in the trial and cured
4. Early withdrawal from treatment and not cured
5. Death from any cause and cured
6. Death from any cause and not cured

4.1.2 Adverse Events and Treatment Outcomes

All patients taking standard TB therapy were grouped according to whether they had achieved microbiological cure as defined above and whether they had experienced ≥1 (total or related-only) grade 3 or 4 AE. These data were tabulated and a Chi square test was used to investigate for statistical significance in the difference in proportions of cure between those patient groups with and without AEs. Binary logistic regression tested the relationship between experiencing ≥1 related grade 3 or 4 AE and achieving eventual cure. This analysis was performed as a univariable regression, and then subsequently as a multivariable model. The multivariable model included sex, age, baseline weight (clinical significance for these three), ethnicity (due to univariable

model significance) and HIV status (due to earlier reported association with AE incidence).

Patients were then categorised as having experienced 0, 1, 2, or ≥3 related grade 3 or 4 AEs on standard therapy. The number of patients in each of these categories was tabulated against the more detailed treatment outcomes to allow for comparison.

4.2 Results

Out of the total 230 grade 3 or 4 AEs reported on the standard arm, 200 (87.0%) were considered to have either improved or recovered (see Table 4-1). This was also the case for events thought to be related to treatment, where 104 of 111 (93.7%) events were classed as recovered or improved by the site doctors. Those events that ended in the patient's death were not included in this count.

	AE OUTCOME				
TOTAL	**IMPROVED**	**ONGOING**	**RECOVERED**	**WORSENED**	**TOTAL**
GRADE 3	57 (32.0%)	21 (11.8%)	98 (55.1%)	2 (1.1%)	178
GRADE 4	23 (44.2%)	7 (13.4%)	22 (42.3%)	0 (0.0%)	52
RELATED	**IMPROVED**	**ONGOING**	**RECOVERED**	**WORSENED**	**TOTAL**
GRADE 3	33 (44.0%)	4 (5.3%)	36 (48.0%)	2 (2.7%)	75
GRADE 4	19 (52.8%)	1 (2.8%)	16 (44.4%)	0 (0.0%)	36

Table 4-1. Reported outcomes for grade 3 or 4 AEs on the standard arm. Total and related-only events are shown, and row percentages included in each cell

While the majority of patients who experienced ≥1 related grade 3 or 4 AEs achieved a microbiological cure this proportion was smaller than seen in the group of patients with no related grade 3 or 4 AEs. 523 of the 582 (89.9%) patients who did not experience a related grade 3 or 4 AE achieved microbiological cure as defined in Section 4.1.1. In comparison 42 of 57 patients (73.7%) who experienced ≥1 related grade 3 or 4 AE achieved cure, as shown in Table 4-2.

	No. of Grade 3/4 AEs	Microbiological Cure	No Microbiological Cure	Total
TOTAL	0	464 (90.8%)	47 (9.2%)	511
	≥1	101 (78.9%)	27 (21.1%)	128
RELATED ONLY	0	523 (89.9%)	59 (10.1%)	582
	≥1	42 (73.7%)	15 (26.3%)	57

Table 4-2. Rates of Microbiological Cure According to Number of Grade 3 or 4 Adverse Events Experienced by Patients Taking Standard TB Therapy. Patients are grouped by the number of AEs they experienced in the trial. The number of patients who were either cured or not cured of their TB are displayed with row percentages (Chi square test p value <0.001)

Patients who experienced ≥1 grade 3 or 4 AE were at significantly higher odds of failing to be cured by their treatment with standard TB therapy. Table 4-3 demonstrates that experiencing ≥1 grade 3 or 4 AE was significantly associated with not being cured of TB in a multivariable logistic regression model (adjOR 2.37, 95% CI 1.37 – 4.14, p<0.01). Table 4-4 also shows a comparable relationship was seen between ≥1 related grade 3/4 AE and an outcome of not cured (adjOR 3.11, 95% CI 1.59 – 6.10, p<0.01).

Baseline Characteristic		OR	95% CI	P value	adjOR	95% CI	P value
≥1 Grade 3 or 4 AE		2.64	1.57 – 4.44	<0.01	2.37	1.36 – 4.14	<0.01
Female Gender		1.13	0.67 – 1.91	0.63	0.97	0.54 – 1.75	0.93
Age*		1.43	1.05 – 1.95	0.02	1.42	1.03 – 1.95	0.03
Baseline Weight*		0.85	0.66 - 1.09	0.20	0.96	0.72 – 1.28	0.76
Ethnicity							
	Black	BASELINE	---	---	---	---	---
	Asian	1.82	1.03 – 3.19	0.04	1.66	0.88 – 3.13	0.12
	Mix Race	1.33	0.69 – 2.58		1.36	0.68 – 2.69	0.38
Smoking Hist.							
	Never	BASELINE	---	---	---	---	---
	Ex-smoker	1.42	0.79 – 2.56	0.24	---	---	---
	Current	1.10	0.61 – 1.97	0.76	---	---	---
HIV Positive		0.93	0.35 – 2.42	0.88	0.85	0.01 – 0.14	0.76
Cavities on CXR		1.17	0.60 – 2.27	0.64	---	---	---
Baseline TTP		1.34	0.50 – 3.57	0.56			

Table 4-3. Output from univariable and multivariable logistic regression with failure to achieve microbiological cure as outcome, with all grade 3 or 4 adverse events included. OR= Odds ratio, adjOR= Adjusted OR, 95% CI=95% confidence intervals. *Age and baseline weight were categorised as presented in Table 3 of this chapter

Baseline Characteristic		OR	95% CI	P value	adjOR	95% CI	P value
≥1 Grade 3 or 4 AE		3.16	1.66 – 6.05	<0.01	2.63	1.30 – 5.35	<0.01
Female Gender		1.13	0.67 – 1.91	0.63	1.00	0.56 – 1.80	0.99
Age*		1.43	1.05 – 1.95	0.02	1.42	1.03 – 1.96	0.03
Baseline Weight*		0.85	0.66 – 1.09	0.20	0.93	0.70 – 1.24	0.61
Ethnicity							
	Black	BASELINE	---	---	---	---	---
	Asian	1.82	1.03 – 3.20	0.04	1.67	0.89 – 3.14	0.11
	Mix Race	1.33	0.69 – 2.58	0.39	1.31	0.67 – 2.61	0.43
Smoking Hist.							
	Never	BASELINE	---	---	---	---	---
	Ex-smoker	1.42	0.79 – 2.56	0.24	---	---	---
	Current	1.10	0.61 – 1.97	0.76	---	---	---
HIV Positive		0.93	0.35 – 2.42	0.88	0.90	0.32 – 2.55	0.85
Cavities on CXR		1.17	0.60 – 2.27	0.64	---	---	---
Baseline TTP		1.34	0.50 – 3.57	0.56	---	---	---

Table 4-4. Output from univariable and multivariable logistic regression with failure to achieve microbiological cure as outcome, with only grade 3 or 4 adverse events assessed as related to treatment included. AE= Adverse events, OR= Odds ratio, adjOR= Adjusted OR, 95% CI=95% confidence intervals. *Age and baseline weight were categorised as presented in Table 3 of this chapter

Table 4-5 displays microbiological outcomes in the trial according to the patient's status, based on whether they completed their treatment without an early withdrawal or died from any cause and the number of related grade 3 or 4 AEs they experienced during the trial. The proportion of patients who completed standard therapy and were cured of their disease can be seen to decrease between the patient group with no events reported (86.6%) down to those patients with ≥3 grade 3 or 4 related AEs (25.0%). However among those patients with ≥1 related grade 3 or 4 AE the majority

(39 of 57, 68.4%) still ultimately reached a microbiological cure, including those patients who were withdrawn early and referred on to the National Treatment Program.

The majority of patients taking standard therapy who died in the trial did not experience any grade 3 or 4 AEs that were thought to be related to therapy. Ten of the sixteen patients did not experience any related event; while only 4 patients and 2 patients experienced 1 and 2 grade 3 or 4 AEs respectively. None of the patients who died experienced ≥3 related grade 3 or 4 AEs, and 9 of the 16 (56.3%) had completed therapy and achieved microbiological cure before dying.

Number Related G3/4 AEs	Completed Treatment		Early Withdrawal from Treatment		Death (Any Cause)		TOTAL
	Cured	Not Cured	Cured	Not Cured	Cured	Not Cured	
0	504 (86.6%)	27 (4.6%)	13 (2.2%)	28 (4.8%)	6 (1.0%)	4 (0.7%)	582 (91.1%)
1	19 (54.3%)	2 (5.7%)	6 (17.1%)	4 (11.4%)	3 (8.6%)	1 (2.9%)	35 (5.5%)
2	4 (28.6%)	1 (7.1%)	4 (28.6%)	3 (21.4%)	0 (0.0%)	2 (14.3%)	14 (2.2%)
≥3	2 (25.0%)	0 (0.0%)	4 (50.0%)	2 (25.0%)	0 (0.0%)	0 (0.0%)	8 (1.3%)
TOTAL	529 (82.8%)	30 (4.7%)	27 (4.2%)	37 (5.8%)	9 (1.4%)	7 (1.1%)	639 (100.0%)

Table 4-5. Microbiological outcomes in the trial according to patient status. Patients are grouped according to the number of related grade 3 or 4 AEs experienced during the trial and whether treatment was completed in the trial or if the patient died at any time from any cause. Row percentages are demonstrated in the cells

5 Incidence of Adverse Events across the three Treatment Arms

The development of new TB drugs will require a balance between efficacy and the safety profile of any regimen to treat the disease in less than six months. In REMoxTB, the isoniazid and ethambutol arms demonstrated a cure rate of approximately 85% and 80% respectively. These are cure rates in keeping with those for some cancers that are now considered “curable”, and in a meta-analysis of the three randomised trials investigating four-month fluoroquinolone-containing regimens patients with a

lower bacillary burden receiving the shorter experimental regimens were found to have non-inferior outcomes compared to the same patients receiving standard TB therapy (Imperial *et al.*, 2018). The safety profile of these experimental regimens could help to further clarify what, if any, role they have in the treatment of TB.

While the experimental arms were not non-inferior to standard therapy, this section characterises the reported toxicity in the experimental arms according to treatment phase and compares this to the standard therapy arm. The intention was to investigate their potential for use in patients at higher risk of side effects from treatment.

5.1 Methods

The number of patients and the total number of grade 3 or 4 AEs in the experimental arms was collected and assigned to different treatment phases based on their start date. The start date of the AEs was used to calculate the number of days after the first drug dose, and the AEs in all three treatment arms were then assigned to one of three time windows: intensive phase (0 – 8 weeks), continuation phase (9 – 17 weeks), or continuation/placebo (17 – 26 weeks). The number of patients reporting ≥1 grade 3 or 4 AE (either total or related-only) in each of the treatment phases was obtained for the three arms. The Chi square test was used to test for significant differences in the proportion of patients reporting ≥1 grade 3 or 4 AE between the treatment arms in each treatment phase.

The time to first related grade 3 or 4 AE was calculated for each patient in the treatment arms, using the **`stset`** command, and Kaplan Meier curves constructed with risk tables. A variable containing the time in weeks since first medication dose was created for each AE using the event start date and the first dose date. The earliest grade 3 or 4 related AE was considered a failure event for each patient. The difference in time to

first AE was tested using the logrank test instead of Cox regression, as visual assessment showed the survival curves crossed and the proportional hazards assumption was violated.

The related grade 3 and 4 AEs were labelled according to their SOC, and totals calculated for each treatment arm. As before, preferred terms were used to group events from the “Investigations” SOC into more clinically relevant SOCs. The events were tallied based on their treatment arm and presented as a bar chart with the SOCs in descending order of frequency.

Finally, the numbers of patients who were withdrawn from treatment or died was investigated in the different arms. All the patients across the treatment arms who were early withdrawals from treatment or deaths from any cause were selected out, and then assigned the event of withdrawal or death to one of the three treatment phases in the treatment arm using its start date. The Kruskal-Wallis test was used to test the variation in timing of withdrawal and timing of death between the three arms as a non-parametric alternative to analysis of variance testing because of a non-normal distribution of the timing data.

5.2 Results

A higher number of grade 3 or 4 AEs occurred in the standard therapy arm compared to the two experimental arms. Patients on the standard therapy arm reported 113 related grade 3 or 4 AEs, compared to 63 & 65 related events in the isoniazid and ethambutol arms respectively. The total number of grade 3 or 4 AEs was similar irrespective of treatment with 250, 217, and 209 events reported across the standard, isoniazid and ethambutol arms as shown in Table 5-1. The number of patients experiencing ≥1 related grade 3 or 3 AE was found to be significantly different in the

intensive phase, with the highest number of patients experiencing events receiving standard therapy, and this phase was when the majority of related grade 3 or 4 AEs occurred in all three treatment arms.

There was no significant difference in the number of grade 3 or 4 AEs occurring between the three treatment arms during Months 5 and 6. There were 2 events reported in the standard and ethambutol arms, and 3 events from the isoniazid arm in this time window. The number of patients mirrored these values; with each patient experiencing only one related grade 3 or 4 AE. 12, 13, and 17 patients reported ≥1 grade 3 or 4 AE in Months 5 and 6 on the standard, isoniazid and ethambutol arms respectively ($p=0.57$). There was no significant difference in the number of patients reporting ≥1 grade 3 or 4 AE ($p=0.81$) or related AE ($p=0.32$) during Months 3 and 4, when all three arms were receiving active treatment (see Table 5-1).

		Standard Arm (2EHRZ/ 4HR) n=639	Isoniazid Arm (2MHRZ/ 2MHR) n=655	Ethambutol Arm (2EMRZ/ 2MR) n=636	P value
Intensive Phase (M0-M2)	**Patients with ≥1 G3/4 AEs (Tot No AEs)**	85 (135)	83 (119)	66 (114)	0.24
	Patients with ≥1 Related G3/4 AEs (Tot No AEs)	47 (80)	36 (51)	25 (44)	0.03
Continuation Phase (M3-M4)	**Patients with ≥1 G3/4 AEs (Tot No AEs)**	29 (47)	25 (32)	26 (37)	0.81
	Patients with ≥1 Related G3/4 AEs (Tot No AEs)	14 (27)	9 (9)	16 (19)	0.32
Cont/Placebo Phase (M5-M6)	**Patients with ≥1 G3/4 AEs (Tot No AEs)**	12 (15)	13 (17)	17 (21)	0.57
	Patients with ≥1 Related G3/4 AEs (Tot No AEs)	2 (2)	3 (3)	2 (2)	0.88
TOTAL*	**Total G3/4 AEs (Related G3/4 AEs only)**	250 (113)	217 (64)	209 (66)	N/A

Table 5-1. Comparing Adverse Events in Treatment Arms. The number of patients experiencing one or more grade 3 or 4 adverse event, and those who experienced events considered related to treatment only, are shown according to the treatment phase and study arm in the trial. The numbers of events are shown in brackets. The Chi square test was used to test for significant differences between the treatment arms for the proportions of patients who experienced ≥1 event in each treatment phase, for both total and related-only grade 3 or 4 AEs. Number of patients shown is number for that treatment window: 4 patients with ≥1 related AE appear in more than one time window on standard therapy, and 3 patients in both the isoniazid and ethambutol arms. Additionally, two patients excluded from the total count on the standard arm as no start date for AEs recorded * AEs that occurred in the follow-up phase (months 7-18) included in total

The time to first related grade 3 or 4 AE was similar in all three treatment arms, with no statistically significant difference detected. The Kaplan Meier curves in Figure 13

illustrate the majority of events occurring in the intensive phase followed by a plateau from approximately 9 weeks after starting treatment (log rank p=0.19 for comparing standard therapy and isoniazid arm; p=0.07 for standard therapy and ethambutol arm). The drop seen at 8 weeks of treatment in the number of patients at risk is driven by one site reporting 40 grade 3/4 AEs (30 considered related) from all treatment arms in a 30-week time window of the trial (the site reported a total of 146 grade 3/4 AEs). 90% (36 of 40) of these events were reported in the intensive phase of the patient's treatment.

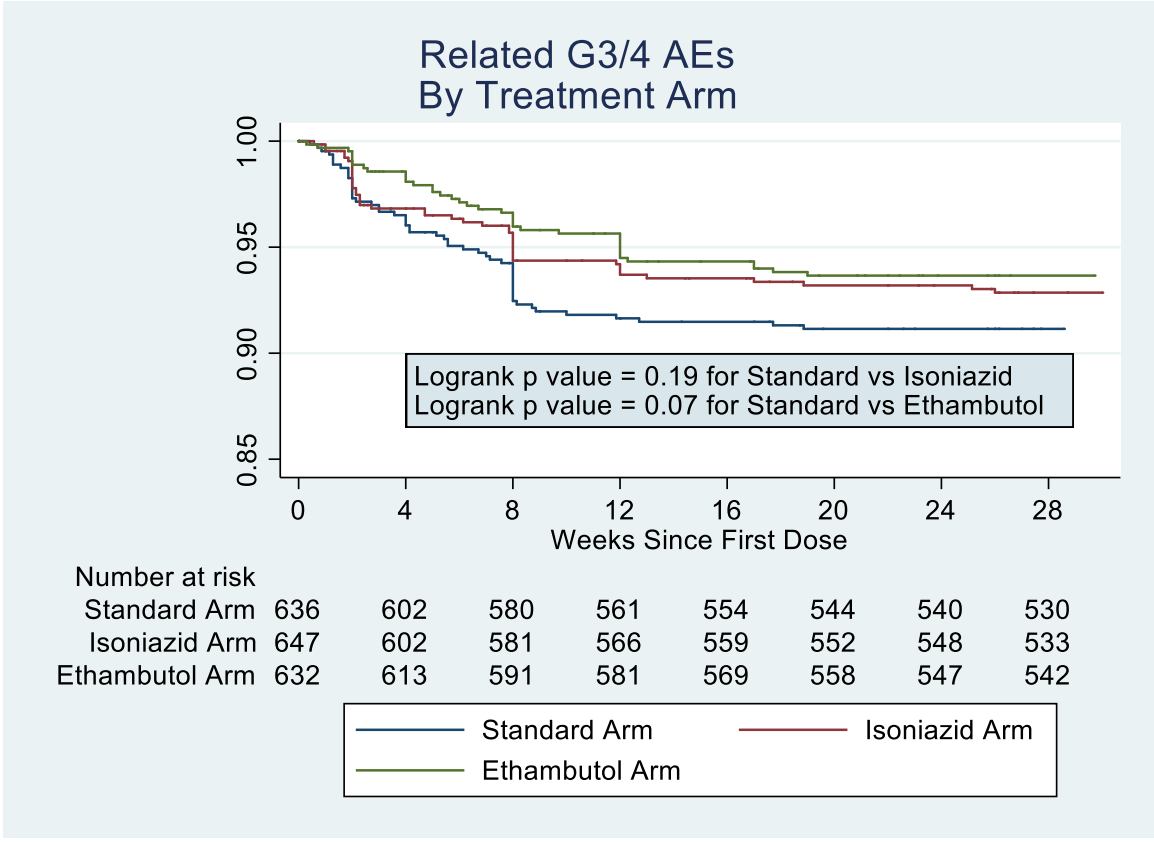

Figure 5-1. Kaplan Meier Curves for time to First Event for Related Grade 3 or 4 Adverse Events in the Treatment Arms. The time to first event is plotted for all the patients at risk in the standard (blue), isoniazid (red) and ethambutol (green) arms

Hepatobiliary disorders were the most common SOC reported for related grade 3 or 4 AEs across all the treatment arms (see Figure 5-2). Within the SOC, the three most common PTs were "Hepatic enzyme increased" (48.1%), "AST increased" (13.5%),

and “ALT increased” (12.5%). Musculoskeletal and connective tissue disorders were the second most common SOC and the two most frequent PTs reported were “Arthralgia” (30.3%) and “Pain in extremity” (21.2%). Standard therapy patients experienced more grade 3 or 4 related AEs than patients allocated to the experimental arms in the five most common SOCs, with the exception of blood and lymphatic disorders. Patients receiving the ethambutol arm experienced the highest number of these events and they were most commonly “Haemoglobin decreased/anaemia” (50.0%) and “Prothrombin time prolonged” (25.0%).

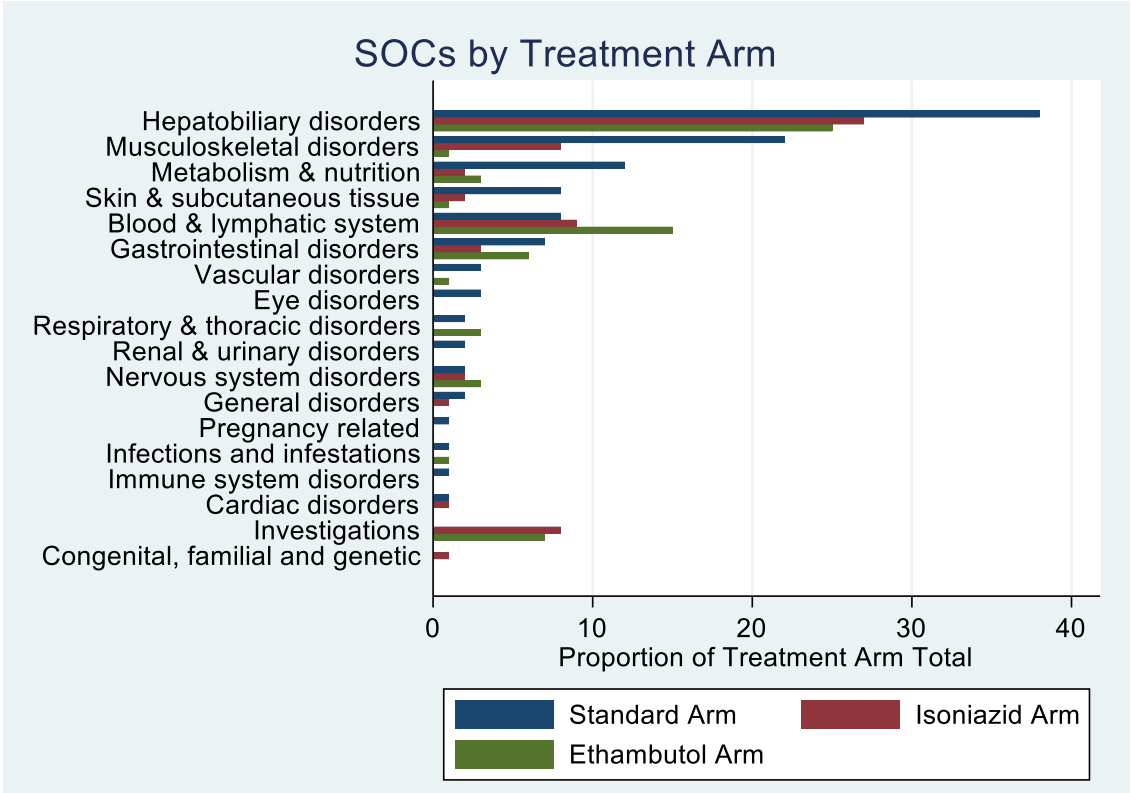

Figure 5-2. Bar chart to illustrate the System Organ Class (SOC) for related grade 3 or 4 AEs in the three treatment arms used for REMoxTB. SOCs are presented in descending order based on the total number of events across the three treatment arms, and then individual counts for the SOC by treatment arm are presented using a colour-coded scheme (as per legend)

The majority of early withdrawals from treatment occurred in the intensive phase for all three treatment arms. Table 5-2 shows that 59.4%, 69.6%, and 50.9% of the withdrawals from the standard, isoniazid and ethambutol arms occurred in the

intensive phase. “Withdrew consent” (25.0%), “Adverse reaction/toxicity” (21.3%), and “MDR/Drug resistance” (15.0%) were the three most common reasons for withdrawal from treatment on the isoniazid arm. On the ethambutol arm, “Protocol violation” (22.4%), “Adverse reaction/toxicity” (20.7%), and “Withdrew consent” (20.7%) were the most frequently reported reasons for withdrawal from treatment. The median timing for withdrawal from treatment was 40 days (IQR 21.0 - 98.5), 34 days (IQR 11.5 – 87.0), and 52 days (IQR 27.0 – 118.0) on the standard, isoniazid and ethambutol arms (p value 0.14 by Kruskal-Wallis with ties).

		2EHRZ/4HR	2MHRZ/2MHR	2EMRZ/2MR
WITHDRAWAL FROM TREATMENT	**Intensive (M0-M2)**	38 (59.4%)	55 (69.6%)	28 (50.9%)
	Continuation (M3-M4)	13 (20.3%)	12 (15.2%)	14 (25.5%)
	Cont/Placebo (M5-M6)	13 (20.3%)	12 (15.2%)	13 (23.6%)
	TOTAL	64	79	55
DEATH	**Intensive (M0-M2)**	5 (31.2%)	5 (33.3%)	3 (25.0%)
	Continuation (M3-M4)	0 (0.0%)	2 (13.3%)	2 (16.7%)
	Cont/Placebo (M5-M6)	1 (6.3%)	2 (13.3%)	3 (25.0%)
	Follow-up (M7-M18)	10 (62.5%)	6 (40.0%)	4 (33.3%)
	TOTAL	16	15	12

Table 5-2. Early withdrawals from treatment and deaths from any cause at any time in each of the three treatment arms. The number of withdrawals in follow up are not shown in this table. Cell percentages refer to proportion of column total

There were fewer deaths reported on both experimental arms compared to the standard therapy arm. On the isoniazid and ethambutol arms there were 15 and 12 deaths, respectively, compared to 16 on standard therapy. There were 6 of 15 (40%) deaths on the isoniazid arm that were due to trauma/violence or unknown cause (but possibly violent) and 4 of 15 (26.7%) deaths related to TB. 5 of 12 (41.7%) deaths on the ethambutol arm were related to TB and only one death was traumatic (a road traffic accident) with no deaths reported as even possibly due to violence. Across the three treatment arms 15 of 43 (34.9%) of deaths were at least possibly due to

violence/trauma, 13 of 43 (30.2%) were due to TB disease, and 15 of 43 (34.9%) were due to other causes (e.g. renal failure, other infections etc). One death on the isoniazid arm was considered possibly related to treatment ("Guillan Barre Syndrome") and 2 were considered possibly related ("hepatitis of unknown cause" and "renal failure") on the ethambutol arm. The median time of death was 35.6 weeks (IQR 5.0 – 45.2), 21.9 weeks (IQR 6.4 – 40.1), and 21.2 weeks (IQR 8.0 – 55.6) on the standard, isoniazid and ethambutol arms (p=0.23 by Kruskal-Wallis with ties).

6 Discussion

This chapter demonstrates that approximately 10% of patients experienced toxicity assessed as being related to standard TB therapy by the treating physician in the REMoxTB trial. This toxicity most commonly took the form of hepatotoxicity, and females and HIV positive patients were significantly more likely to experience clinically significant adverse events while taking treatment. The two experimental arms in the trial reported lower numbers of adverse events, suggesting they are less toxic treatment options, but this must be balanced against their failure to achieve non-inferiority when compared to standard therapy treatment outcomes.

The existing literature quotes rates of approximately 1 - 40% for patients experiencing toxicity related to therapy (Saukkonen *et al.*, 2006; Marra *et al.*, 2007; Kumar *et al.*, 2010; Devarbhavi, 2011; Lorent *et al.*, 2011; Zhang *et al.*, 2015; Njuguna *et al.*, 2016; Yang *et al.*, 2016; Sekaggya-Wiltshire *et al.*, 2017), however most commonly these publications are observational or retrospective and related to hepatotoxicity. The estimate of approximately 10% of patients experiencing toxicity related to treatment is the product of detailed safety reporting in a large, prospective trial and while some confidence can be drawn from the data collection methods it still needs to be remembered that this is essentially a cohort study and can therefore still be subject to

bias. Two further caveats are that the proportion of patients experiencing any significant AE was 20%, closer to the upper limit to the range in the existing literature, and that the relatedness assessment is of course subject to bias on the part of the physician carrying out the assessment (Hillman *et al.*, 2017).

The majority of clinically significant adverse events occurred in the intensive phase of treatment with implications for the routine monitoring of patients taking standard TB therapy. Pyrazinamide could account for most of the related grade 3 or 4 AEs in standard therapy occurring in the intensive phase, although this is far from certain. Pyrazinamide is a drug with a well-recognised toxicity profile (Horsfall *et al.*, 1979; Jenner *et al.*, 1981; Steele and Des Prez, 1988; Schaberg, Rebhan and Lode, 1996; Jasmer *et al.*, 2002; Lee *et al.*, 2002; Yee *et al.*, 2003), while ethambutol (the other drug only present for the intensive phase) is recognised as having fewer side effects. Additionally, hepatotoxicity and arthralgia were among the most common events and these are frequently reported side effects of pyrazinamide. The sterilising activity of pyrazinamide makes it an essential component of standard therapy (British Medical Research Council, 1974; Grosset, 1978; Association, 1982; Snider *et al.*, 1984), but there is still some uncertainty surrounding its ideal dosing schedule (British Medical Research Council, 1981; Hong Kong Chest Service/Tuberculosis Research Centre, 1989; Jindani, Nunn and Enarson, 2004; Chang *et al.*, 2007) and there is evidence of both a dose-response relationship with toxicity (Service, 1959) and also idiosyncratic drug-induced liver injury (Pasipanodya and Gumbo, 2010). There is a need to direct more research to optimise the most effective and least toxic dose alongside the other components of the standard regimen.

HIV positive patients, female patients, and patients with a lower weight at baseline were shown to be at risk for related clinically significant adverse events, and this

should be reflected in clinical practice. While existing guidelines acknowledge the issues surrounding the management of TB-HIV co-infection (Saukkonen *et al.*, 2006; World Health Organization, 2010; Nahid *et al.*, 2016), there is no reference to female gender as a risk factor for a more complicated treatment course (outside of pregnancy). There is some evidence in the literature regarding higher observed rates of toxicity for both males and females (Marra *et al.*, 2007; Pettit *et al.*, 2013; Sadiq *et al.*, 2015; Zhang *et al.*, 2015), and subsequent work on treatment outcomes by gender has shown that males with cavitation on the ethambutol arm in REMoxTB had especially poor outcomes (Murphy ME et al, in review). However, it is unclear if reporting bias has played a role in AE recording for the trial (McGauran *et al.*, 2010) and the likelihood for patients themselves to attribute any symptoms to the medication: previous work has identified discrepancies between males and females in regards to healthcare-seeking behaviour (Samal, 2016; Thompson *et al.*, 2016; Hawkins *et al.*, 2017). Nonetheless, clinicians should have a low threshold for closer monitoring of female patients, those with a low weight at baseline, and HIV positive patients taking standard TB therapy.

Diabetes and its complications accounted for a large proportion of the adverse events classed as “Metabolism and Nutrition Disorders” in this analysis. It is projected that by 2040 there will be approximately 640 million people with either diagnosed or undiagnosed diabetes (International Diabetes Federation, 2017), and the majority of these will be found in low- and middle-income countries (Dooley and Chaisson, 2009; International Diabetes Federation, 2017). In light of this, there has been growing interest in the influence of diabetes on TB over the past decade (Dooley and Chaisson, 2009). Meta-analyses of existing publications relating to TB and diabetes have shown a relative risk of 3.1 for developing TB in diabetic patients (Jeon and Murray, 2008)

and also an odds ratio of 1.69 for an unfavourable treatment outcome (treatment failure or death) in diabetic patients compared to non-diabetic patients(Baker *et al.*, 2011). As well as diabetes leading to increased risk of active TB disease, there is an effect of active TB on glucose homeostasis. Several studies have demonstrated impaired glucose tolerance among patients with TB (Nichols, 1957; Zack, Fulkerson and Stein, 1973), but this most commonly returns to normal within 3 months of completing treatment (Zack, Fulkerson and Stein, 1973; Oluboyo and Erasmus, 1990). Whether the patients reported as diabetic in REMoxTB had a true diagnosis or a transient loss of blood glucose control which then normalised is unclear, yet the prevalence of AEs in this chapter that are related to glucose dysregulation reinforces the need for coordinated care between physicians specialising in TB and diabetes. The best approach to screening patients with diabetes for the presence of either latent or active TB remains uncertain (World Health Organization and the International Union Against Tuberculosis and Lung Disease., 2011); however, the fact that 35% of the "Metabolism and Nutrition" adverse events (the third most common SOC among patients on standard TB therapy) involved diabetes or dysglycaemia lends weight to the argument that a co-ordinated approach between between TB physicians and diabetologists to screening and management is a area that merits further attention.

This chapter has shown that patients who experienced one or more clinically significant adverse event were more likely to fail their TB treatment, compared to patients who did not experience a clinically significant event. The reasons for this association are not clear and should prompt further investigation. It may be that this is due to other underlying conditions that also contribute to a poor outcome or the effect of toxicity on adherence to therapy. Patients in REMoxTB were managed under clinical trial conditions with approximately 90% of patients achieving cure (Gillespie *et al.*,

2014), however it is recognised that the cure rate with HRZE for drug-sensitive disease can fall to approximately 80% in real-world settings (WHO, 2017) and the relationship demonstrated here may help explain this finding.

Treatment interruption is another possible explanation for the association between grade 3 and 4 AEs and the increased odds of failing treatment. Most of the clinically significant AEs were reported as either resolved or improved as their outcome, suggesting that the event did not have persisting sequelae that directly led to a poor treatment outcome. However, treatment interruptions have a recognised association with poor outcomes for TB patients (Ormerod and Prescott, 1991; Pablos-Méndez *et al.*, 1997; Chee *et al.*, 2000) and it is conceivable that medication would have been withheld in the context of events that were thought to be either related or unrelated to the treatment due to symptoms. Unfortunately, the medication adherence data was not adequately recorded by the sites during the trial to allow it to be used in this analysis: despite adherence data being available for over 90% of randomised study participants, there were inconsistent entries in many cases and the trial data scientists used either simple deduction after manual review or automated imputation methods to complete the dataset (exact number of participants data affected was unavailable). Additionally, there were concerns raised at several sites regarding the source documentation for medication adherence (although not for other source documentation). Following internal discussion, it was felt that the dataset could be used to assess whether participants overall adherence was greater than the threshold of 80% adherence but it could not be relied upon to accurately relate discrete episodes of toxicity to medication interruptions.

There is some indication that the experimental regimens in REMoxTB could have a role as less toxic treatment options for use in patient groups deemed to be “high-risk”

by the treating clinician. The evidence presented in this section demonstrates a lower rate of adverse events in the two experimental arms, but this needs to be balanced against their failure to achieve non-inferiority compared to standard TB therapy for treatment outcome (Gillespie *et al.*, 2014). While the experimental arms failed to achieve non-inferiority compared to standard TB therapy in trial conditions, the cure rates achieved with standard TB therapy in the field are closer to 80% (WHO, 2017) and this is very similar to the cure rates seen in the isoniazid and ethambutol arms. Furthermore, the optimal duration of the experimental regimens is not clear and they could perhaps show improved rates of cure if carried on to six months with lower rates of toxicity than standard therapy. This is plausible given that the majority of AEs occurred in the first two months on all three treatment arms.

There was little difference in the number of related AEs in months 5 and 6 between those receiving active treatment and those on placebo. This does, perhaps, emphasise the importance of TB induced pathology on the presence and reporting of significant medical events. Reducing toxicity associated with medication is one of the factors driving the development of shorter treatment regimens for TB (Zumla *et al.*, 2014); however, this finding suggests that concerns about toxicity may not be as important as previously thought. Again, it should be highlighted that while the experimental arms were less toxic, they were also less effective. Whether there is a causal relationship between these observations is not known. This could mean that the motivation for shortening treatment needs to focus around patient acceptability and logistical benefits of few doses, a reduced number of clinic appointments, and enhanced adherence.

6.1 Limitations

This study is limited by innate reporting bias and reliance on a subjective assessment of severity in many cases (for example, pain scores). An example of this is the reporting activity at one site in the trial. After a trial pause this site reported almost one third of its total grade 3/4 AEs, and assessed 75% of them as being related to treatment in a 30-week period. Attributing causality to AEs has been shown to produce unreliable and subjective data (Hillman *et al.*, 2017) and caution has been advised when using trial data to evaluate drug safety profiles (Hammad, Pinheiro and Neyarapally, 2011). Given the proximity of the recent pause in trial recruitment it could be that there was concern over the safety of the experimental regimens and that in a double-blind trial this translated into a lower threshold to both report events and to attribute causality to the drugs.

While there is still merit in using AEs to investigate drug safety profiles, the often subjective nature of the reporting is a limitation. This analysis focusses on clinically significant adverse events (grade 3 or 4 severity) associated with tuberculosis therapy as symptoms were not captured. There are also the potential dangers of drawing conclusions based on relatedness assessments for AEs (Darssan, Thompson and Pettitt, 2014; Hillman *et al.*, 2017) and to this end both total and related AEs in the analysis have been presented.

6.2 Conclusion

The work presented in this chapter has shown that most adverse events occur during the intensive phase of treatment with female patients, those patients with a lower baseline weight and those who are HIV positive constituting a demographic that is most at risk of adverse events. Additionally, those who experienced clinically

significant drug related-toxicity while taking standard TB therapy were at greater risk of failing treatment. From this it can be seen that there is a need to improve our methods of detecting and managing patients experiencing toxicity, and that there is real need for novel drugs with more favourable toxicity profiles and preserved efficacy in treating active pulmonary TB. These data provide an evidence base to plan future research and to support improved treatment guidelines. Tuberculosis remains a global health threat, predominantly affecting a vulnerable and disadvantaged population, and this chapter illustrates the need for clinicians to be quick to respond to side effects from treatment to ensure their patients have the best chance of achieving a cure.

Chapter Four: Liver Toxicity during Tuberculosis Treatment

1 General Introduction

Hepatotoxicity was shown in the previous chapter to be the most common form of toxicity during treatment with standard tuberculosis (TB) therapy, and this chapter will characterise the pattern of liver enzyme elevations on treatment in more detail. Liver enzyme elevations and drug-induced liver injury (DILI) are recognised side effects of standard TB therapy. The reported rates of hepatotoxicity on standard therapy vary between 5-30% (Thompson *et al.*, 1995; Tost *et al.*, 2005; Saukkonen *et al.*, 2006; Shu *et al.*, 2013), and this range is related to the variable definitions used in the literature (Devarbhavi, 2012; Saukkonen, Powell and Jereb, 2012). For example, pre-existing liver disease may be defined as elevated liver enzyme levels at baseline (Ormerod *et al.*, 1998; Saukkonen *et al.*, 2006), previous hepatitis B or C infection (Ungo *et al.*, 1998; Kwon *et al.*, 2007; De Castro *et al.*, 2010; N.-T. Wang *et al.*, 2016), or established cirrhosis.

Hepatotoxicity has been associated with treatment interruptions, increased patient morbidity, and poorer treatment outcomes to greater or lesser degrees (Shang *et al.*, 2011; Pandit, Sachdeva and Bafna, 2012; Seaworth, Armitige and Griffith, 2013; Chou *et al.*, 2014). Current guidelines for the monitoring of patients taking standard TB therapy and the management of liver enzyme elevations on treatment are largely based on expert opinion. The British Thoracic Society (BTS) guidelines recommend treatment interruption when ALT or AST are elevated to five times the upper limit of

normal or when bilirubin rises above the upper limit of normal (Ormerod *et al.*, 1998). The American Thoracic Society (ATS), in contrast, recommends interruption when hepatic enzymes are greater than three times the upper limit of normal with symptoms or jaundice, or more than five times the upper limit of normal in asymptomatic patients (Saukkonen *et al.*, 2006). Furthermore, the European Respiratory Society (ERS) recommends withholding potentially hepatotoxic medications (H, R, and Z) if transaminases are greater than five times the upper limit of normal (Migliori *et al.*, 1999). However, the timing and magnitude of elevations in the serum transaminases over the course of treatment in the entire patient population, with and without symptoms, and the outcome of treatment-related enzyme changes, remain unclear. This makes the appropriate management of liver toxicity, including timing of treatment interruption and re-introduction uncertain (Saukkonen, 2010).

In 2011 the DILI Expert Working Group published a consensus statement to define criteria for use in the diagnosis of “true” DILI (Aithal *et al.*, 2011). Their motivation was to produce guidance that could be applied in both routine clinical practice and in trial settings to increase the chance of a drug’s hepatotoxic potential being identified early, prevent unnecessary treatment interruptions, and avoid delays in reaching the correct diagnosis where liver enzyme elevations could potentially distract from the actual underlying pathology. In this document DILI is defined as any of the following: alanine aminotransferase (ALT) $\geq$5x the upper limit of normal (ULN), alkaline phosphatase levels $\geq$2xULN, or ALT $\geq$3xULN with simultaneous bilirubin >2xULN.

Pre-existing liver disease, HIV infection, female gender, increasing age, and alcohol excess have previously been linked with an increased risk of clinically significant hepatotoxicity during treatment (Teleman *et al.*, 2002; Yee *et al.*, 2003; Fauzi *et al.*, 2004; Fernández-Villar *et al.*, 2004; Chou *et al.*, 2014; Hosford *et al.*, 2014; Yimer *et*

al., 2014). Whether a patient is considered to be a “slow acetylator” based on their NAT2 genotype has also been linked to a higher risk of hepatotoxicity when taking regimens containing isoniazid (Huang *et al.*, 2002; Ng *et al.*, 2014).

In the REMoxTB trial (Gillespie *et al.*, 2014) liver biochemical tests were performed at regular intervals throughout treatment irrespective of the clinical picture. This presents an opportunity to accurately characterise the patterns of enzyme elevations seen in a complete population of patients taking standard TB therapy. This chapter will investigate these patterns with the following research questions:

- What was the distribution of peak liver enzyme results for all patients taking standard TB therapy?
- What was the timing of peak liver enzyme results on standard TB therapy?
- Which patient groups were at greater risk of clinically significant elevations?
- What is the association between significant liver elevations and treatment outcome?
- What was the pattern of peak liver enzyme results on the experimental regimens?
- What was the effect of individual drugs in the regimen on peak enzyme results?

2 Magnitude of Peak Liver Enzyme Elevations on Standard TB Therapy

The pattern of peak liver enzyme elevations, with and without symptoms, experienced by patients taking standard TB therapy is not accurately known. The majority of studies available are retrospective and observational, and relate to clinically apparent episodes of hepatotoxicity that are then confirmed with biochemical tests (Shang *et al.*, 2011; Wu *et al.*, 2012; Chou *et al.*, 2014; Abbara *et al.*, 2017). Knowledge of the

pattern of liver enzyme elevations in asymptomatic patients would provide clinicians with an evidence-based framework to guide decisions to withhold treatment and hopefully reduce the number of unnecessary interruptions to TB treatment.

This section addresses the magnitude of enzyme elevations detected for all patients taking standard TB therapy in REMoxTB. Total bilirubin and international normalised ratio (INR) results, and cases of DILI are also described along with the symptom profile for those patients with clinically significant elevations.

2.1 Methods

Patients were included in the analysis reported in this chapter if they had received at least one dose of TB treatment and had liver biochemical tests (LBT) measured two times or more, with at least one measurement after the first dose of medication. LBT results from between fourteen days before the first dose of medication and up to seven days after the last dose of trial medication were eligible for the analysis. All LBT results are presented as multiples of the ULN to account for variations in the normal range for the tests at the local laboratories.

2.1.1 Peak ALT and AST Results for all Patients

The peak ALT and AST values for all patients suitable for inclusion in the analysis were obtained from the dataset. The distribution was assessed using histograms and inverse normal plotting, and the median values for the peak ALT and AST for all patients taking standard TB therapy was determined.

2.1.2 Drug-induced Liver Injury and Clinically Significant Liver Enzyme Elevations

In this analysis DILI was defined as either ALT ≥5xULN or ALT ≥3xULN with total bilirubin >2xULN, based on the 2011 international DILI Expert Working Group

consensus statement (Aithal *et al.*, 2011). All ALT elevations ≥3xULN (with or without elevated bilirubin or INR) were considered “clinically significant” based on the thresholds used by both the BTS and ATS guidance (Ormerod *et al.*, 1998; Saukkonen *et al.*, 2006). Patients were then categorised according to their peak ALT result based on these definitions.

Data relating to symptoms were obtained from the trial adverse event reporting system. All events recorded within a time window of seven days before until seven days after the peak ALT result were collected and reviewed for patients who experienced either DILI or clinically significant ALT elevations. Those events belonging to gastrointestinal or hepatobiliary System Organ Classes were then manually reviewed and any containing information on symptoms were flagged and totalled.

2.1.3 Additional Hepatotoxic Factors and Causality Assessment for Liver Enzyme Elevations

The Serious Adverse Event (SAE) narratives for the REMoxTB trial for all patients with a peak ALT ≥3xULN who had experienced one or more SAE were reviewed for information regarding treatment interruptions, herbal medications, alcohol use and additional tests that were carried out. Additionally, where sufficient information was available, the Roussel-Uclaf Causality Assessment Method (RUCAM) (Benichou, Danan and Flahault, 1993; Danan and Benichou, 1993) was applied to test for evidence of a causal relationship between standard TB therapy and a peak ALT ≥3xULN.

A table detailing the criteria used to apply the score can be found in Appendix 2. The calculated score can range from -9 to +14 and a score of 0 or less indicates that the drug is “excluded”, scores of 1-2 means it is “unlikely” to be the cause, 3-5 that it is

"possible" it is the cause, 6-8 that is "probable", and finally scores of greater than 8 mean the drug is considered as "highly probable" in terms of a causal relationship to any liver dysfunction

2.1.4 Baseline Liver Disease and Liver Enzyme Elevations

Baseline liver disease and its relationship to liver elevations on treatment was explored by identifying patients with evidence of Child-Pugh disease A or B (Cholongitas *et al.*, 2005), and those patients with baseline ALT >1xULN. A regular expression search was carried out for the terms "ascites", "cirrhosis", "child", "hepatic", and "liver" in the adverse event records. The Child-Pugh scoring criteria, as detailed in Appendix 2, was applied to those patients with evidence of chronic liver disease within one month of their first dose of trial medication to categorise those with stage A or B liver disease.

2.2 Results

A total of 639 patients from the standard therapy arm were eligible for inclusion in this analysis. There were 640 patients initially randomised into the standard therapy arm but one patient did not receive any medication.

2.2.1 Peak ALT and AST Results for all Patients Receiving Standard TB Therapy

The median peak ALT value was 0.83xULN (IQR 0.56 – 1.35) and the median peak AST value was 1.02 (IQR 0.73 – 1.48) for all patients taking standard TB therapy. The histograms in Figure 2-1 show the distribution of the peak liver enzyme results and these were not normally distributed on the inverse normal plots.

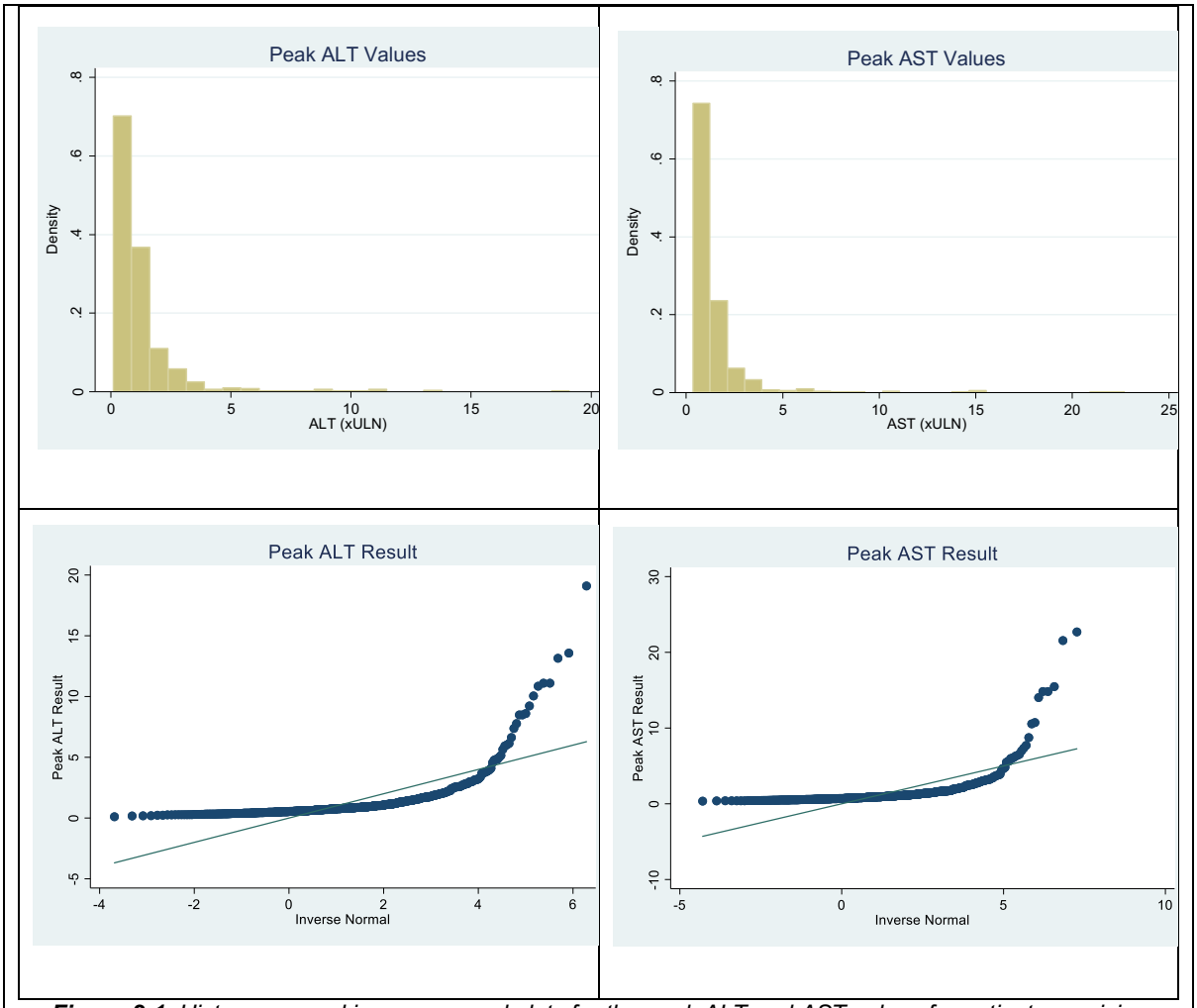

Figure 2-1. *Histograms and inverse normal plots for the peak ALT and AST values for patients receiving standard TB therapy*

The median value for both ALT and AST at protocol scheduled visits is shown in Figure 2-2, where it can be seen that the median AST result had a slight upward trend (from a median value of approximately 0.6xULN to 0.7xULN) during treatment and was consistently higher than the median ALT at every visit.

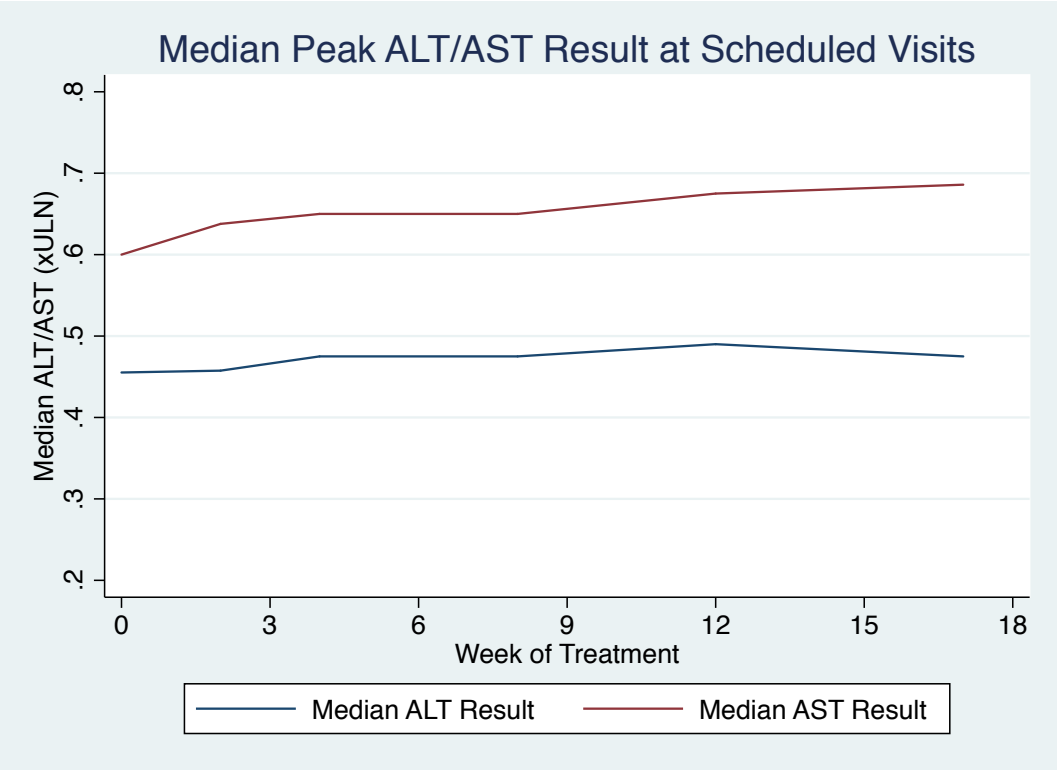

Figure 2-2. *Median peak ALT and AST results at protocol scheduled visits as xULN*

2.2.2 Drug-induced Liver Injury and Clinically Significant Liver Enzyme Elevations

There were 22 of 639 (3.4%) patients taking standard TB therapy who met the criteria for DILI as defined in Section 2.1.2. Among these 22 patients, 8 patients reported diarrhoea and/or vomiting, 10 reported nausea, and 3 reported abdominal pain within two weeks of their peak ALT value.

In total, 41 of 639 (6.4%) patients experienced clinically significant peak ALT elevations during treatment with standard TB therapy. There were 4 patients who developed diarrhoea and/or vomiting, and 5 who reported nausea around the time of their peak ALT.

2.2.3 Additional Hepatotoxic Factors and Causality Assessment for Liver Enzyme Elevations on Standard TB Therapy

Viral hepatitis serology indicating acute hepatitis B infection was detected in one case of DILI on the standard arm. Excess alcohol use was reported in 3 patients with clinically significant peak ALT results. There were no reported cases of herbal medication use among patients receiving standard TB therapy. The RUCAM scoring system was applied to 7 of the 22 (31.8%) patients who experienced DILI and had sufficient information available from their SAE narrative with a median result of 8 (IQR 3-8).

2.2.4 Baseline Liver Disease and Liver Enzyme Elevations

At the time of randomisation there were 65 of 639 (10.2%) patients with a measured ALT result >1xULN and <3xULN. 4 of the 65 (6.2%) patients with baseline elevation went on the experience DILI during treatment, as compared to 22 of 639 (3.4%) in the treatment arm overall. There were no patients receiving standard TB therapy who had evidence of chronic liver disease at enrolment based on the applied search criteria in Section 2.1.4.

3 Timing of Peak Liver Enzyme Elevations on Standard TB Therapy

Just as the magnitude of enzyme elevations on standard TB therapy is not accurately known, neither is the timing of peak enzyme elevations. While clinically significant episodes of hepatotoxicity have been predominantly described early in treatment (Singanayagam *et al.*, 2012; Abbara *et al.*, 2017), it is unclear when peak liver enzyme elevations are to be expected in all patients regardless of the clinical picture. Such information, together with the expected pattern of the elevations would be helpful for

the development of monitoring programs designed to detect significant liver enzyme elevations.

This is particularly true in resource-limited settings where it is critical that services are used cost-effectively. In this section the timing of the peak enzyme elevations for all patients, and those with DILI or clinically significant elevations will be described. The aim is to characterise when elevations are most commonly seen to identify the optimum time windows to be included in patient monitoring programmes.

3.1 Methods

The time in days from first dose of medication to peak ALT result was calculated for each patient receiving standard TB therapy. Peak liver enzyme results that occurred before the first dose of medication were considered to have occurred at day zero. The time to return to within the normal range from peak value was calculated for patients with a peak ALT and/or AST >1xULN and ≥3xULN.

3.2 Results

The median time to reach the peak ALT value was 28 days (IQR 14 – 84) among all patients taking standard TB therapy. The median time to reach the peak AST value was 53 days (IQR 14 – 84). The peak ALT result was reached at a median of 21 days (IQR 14 – 56) for those patients who met the criteria for DILI, and 28 days (IQR 14 – 56) in those patients with a clinically significant peak ALT.

The median time to return to within normal range was 20.5 days (IQR 14.5 – 38.5) for patients with DILI. Those patients with clinically significant peak ALT results (but less than 5xULN) returned to within the normal range at a median of 28 days (IQR 14 – 42). Patients with a peak ALT result >1xULN and <3xULN took a median of 28 days (IQR 15.5 – 35) to return to within normal range from their peak value. Table 3-1

summarises the time to peak ALT and time to normalisation for patients taking standard TB therapy based on their peak ALT result.

	Peak ALT >1xULN & <3xULN	Peak ALT ≥3xULN & <5xULN	DILI (ALT >5xULN or ALT ≥3xULN & Bilirubin >2xULN)
Median Time to Peak ALT in Days (IQR)	25 (10 – 56)	28 (14 – 56)	21 (14 – 56)
Median Time to ALT <1xULN in Days (IQR)	28 (15.5 – 35)	28 (14 – 42)	20.5 (14.5 – 38.5)

Table 3-1. *Time to peak ALT value and time to return to within normal range from peak ALT value displayed according to the magnitude of the peak ALT result. Time given as median number of days with interquartile range (IQR) included in the cells*

4 Patient Characteristics and Liver Enzyme Elevations on Standard TB Therapy

There is a lack of clarity in the existing literature as to the true extent of the risk for hepatic dysfunction according to patient characteristics. This can be attributed to a combination of varying definitions of hepatotoxicity, different monitoring approaches, and a sometimes unclear prevalence of risk factors in largely observational studies. The extent of this risk has been poorly quantified due to the limitations of the study design even among those patient populations with clearly increased risk of hepatotoxicity.

The identification of those patients at greatest risk of significant hepatotoxicity would allow clinicians to channel their resources and ensure that these vulnerable patients are managed appropriately according to a clear idea of the true extent of this risk. Baseline characteristics for patients in the REMoxTB trial were used in regression analysis to identify which patient groups were at significantly higher risk of liver enzyme elevations while taking standard TB therapy.

4.1 Methods

Patients receiving standard TB therapy were grouped based on their peak ALT or AST result, into either a peak liver enzyme value of <3xULN or ≥3xULN, and the results tabulated according to the baseline characteristics to allow for visual comparison. Logistic regression was used to test for a significant association between baseline characteristics and binary outcomes of clinically significant peak ALT or DILI separately. Univariable regression was manually performed for each of the baseline characteristics against the binary outcome variables of DILI or clinically significant ALT elevation. Variables with a univariable p value of <0.10 were selected for inclusion in the multivariable model along with sex, age, and ethnicity as these were thought to be clinically significant regardless of their p value.

Patients with “extreme” ALT elevations (>10xULN) were selected and more detailed scrutiny of the clinical picture at the time of the elevation was carried out using SAE narratives and adverse events within two weeks before or after the elevation. The site doctor was required to submit a narrative to accompany a SAE at the time of reporting the event. This narrative would include the clinical history of the event including the timelines for symptoms or key aspects of the SAE (as judged by the site doctor), results for any investigations carried out as part of the SAE that were not recorded in the trial database (e.g. additional virology screening or abdominal ultrasound), and any additional background history (such as alcohol excess). These were manually reviewed to gain an understanding of the clinical presentation, contributing factors identified, and any other information thought to be of relevance. While there were mandatory fields (including time of onset, severity grading, relatedness etc), the free text of the narrative was largely left to the site doctor to complete and therefore subject to their bias towards what constituted an important element of the history.

4.2 Results

4.2.1 Clinically Significant Liver Enzyme Elevations on Standard TB Therapy

On standard TB therapy the three categories of patients with the highest proportion of clinically significant peak liver enzyme results were those with a baseline weight less than 40kg, patients of Asian ethnicity, and patients aged 55 years or older at baseline (see Table 4-1). Of these categories, those aged 55 or over at baseline contained the highest proportion of patients reaching a peak liver enzyme result of ≥3xULN (21.1% vs 2.3% to 11.4% in the other categories). Patients who were HIV positive also had a higher proportion of peak ALT and/or AST results ≥3xULN compared to patients who were HIV negative (15.2% vs 8.9%).

		Peak ALT/AST <3xULN	Peak ALT/AST ≥3xULN	TOTAL
n (%)		579 (90.6%)	60 (9.4%)	639
Male Gender (%)		407 (91.1%)	40 (9.0%)	447
Female Gender (%)		172 (89.6%)	20 (10.4%)	192
Age in Years (%)				
	18-24	173 (93.0%)	13 (7.0%)	186
	25-34	163 (88.6%)	21 (11.4%)	184
	35-44	126 (88.7%)	16 (11.3%)	142
	45-54	86 (97.7%)	2 (2.3%)	88
	≥55	30 (78.9%)	8 (21.1%)	38
Baseline Weight in Kg (%)				
	<40	52 (82.5%)	11 (17.5%)	63
	40-49	196 (89.9%)	22 (10.1%)	218
	50-59	226 (93.4%)	16 (6.6%)	242
	60-69	80 (90.9%)	8 (9.1%)	88
	≥70	25 (89.3%)	3 (10.7%)	28
Ethnicity (%)				
	Black	275 (93.2%)	20 (6.8%)	295
	Asian	160 (82.5%)	34 (17.5%)	194
	Mix. Race	143 (96.0%)	6 (4.0%)	149
	Other	1 (100.0%)	0 (0.0%)	1
HIV Status (%)				
	Positive	39 (84.8%)	7 (15.2%)	46
	Negative	540 (91.1%)	53 (8.9%)	593
Cavities on CXR (%)		422 (92.5%)	34 (7.5%)	456
Smoking History (%)				
	Never	267 (89.6%)	31 (10.4%)	298
	Previous	144 (92.9%)	11 (7.1%)	155
	Current	168 (90.3%)	18 (9.7%)	186

Table 4-1. *Patient baseline characteristics categorised to display proportions with a peak ALT and/or AST <3xULN and ≥3xULN. Row percentages included in each cell with row totals provided in far-right column. Data was not available for all patients regarding their date of birth (approximate age used where possible) or for cavitation on chest X-ray*

4.2.2 Baseline Characteristics and Liver Enzyme Elevations

Asian ethnicity was the only baseline characteristic found to be significantly associated with the onset of DILI during treatment. This demographic was significantly associated with DILI in both univariable logistic regression (crude OR 4.86, 95% CI 1.74 – 13.60) and when included in a multivariable model controlling for age and gender (adjOR 4.82, 95% CI 1.72 – 13.51). Female gender and HIV positive status were not significantly associated with DILI in this model. Table 4-2 details the output from both

univariable and multivariable logistic regression of baseline characteristics against the outcome of DILI, and it can be seen that no other characteristics were found to have a significant relationship to DILI during treatment with standard TB therapy.

		Crude OR	P value	95% CI	Adj OR	P value	95% CI
Age (Years)†		1.01	0.70	0.97 – 1.04	1.00	0.82	0.97 – 1.04
Gender							
	Male	BASELINE	---	---	BASELINE	---	---
	Female	1.09	0.85	0.44 – 2.72	1.09	0.85	0.43 – 2.80
Baseline Weight†		0.77	0.26	0.49 – 1.21	---	---	---
Ethnicity							
	Black	BASELINE	---	---	BASELINE	---	---
	Asian	4.86	<0.01	1.74 – 13.60	4.82	<0.01	1.72 – 13.51
	Mixed	0.84	0.84	0.16 – 4.39	0.84	0.83	0.16 – 4.36
HIV Status							
	Negative	BASELINE	---	---	---	---	---
	Positive	1.30	0.73	0.29 – 5.75	---	---	---
XR Cavities							
	None	BASELINE	---	---	---	---	---
	Present	0.51	0.19	0.19 – 1.39	---	---	---
Smoking							
	Never	BASELINE	---	---	---	---	---
	Previous	0.96	0.94	0.32 – 2.86	---	---	---
	Current	1.13	0.81	0.42 – 3.01	---	---	---

Table 4-2. *Logistic regression of baseline characteristics as exposure variables against an outcome of DILI during treatment. Univariable odds ratios (crude) and multivariable odds ratios (adjusted) shown, with a univariable p value <0.10 as inclusion criteria for multivariable model. †Analysed as categorical variable*

Similarly to the findings for DILI, Asian ethnicity was also associated with clinically significant peak ALT results while undergoing treatment with standard TB therapy (adjOR 3.89, 95% CI 1.68 – 9.04, p value <0.01). Cavitation on chest X-ray was associated with reduced odds of experiencing a clinically significant peak ALT result in a univariable analysis (crude OR 0.45, 95% CI 0.22 – 0.94), and this effect persisted into the multivariable model although the p value fell just below the threshold for significance (adjOR 0.50, 95% CI 0.23 – 1.06). Female gender and HIV infection again failed to achieve statistical significance, at the 5% level cut-off specified in Section 2 of the Methods chapter, in either a univariable or multivariable analysis. Table 4-3 contains the results from both the univariable and multivariable logistic regression of baseline characteristics against the binary outcome of a clinically significant peak ALT.

		Crude OR	P value	95% CI	Adj OR	P value	95% CI
Age (Years)†		1.01	0.34	0.99 – 1.04	1.00	0.73	0.98 – 1.03
Gender							
	Male	BASELINE	---	---	BASELINE	---	---
	Female	1.37	0.35	0.71 – 2.65	0.95	0.90	0.43 – 2.09
Baseline Weight†		0.82	0.24	0.58 – 1.14	---	---	---
Ethnicity							
	Black	BASELINE	---	---	BASELINE	---	---
	Asian	3.82	<0.01	1.83 – 7.98	3.89	<0.01	1.68 – 9.04
	Mixed	0.96	0.94	0.33 – 2.81	1.29	0.66	0.41 – 4.06
HIV Status							
	Negative	BASELINE	---	---	---	---	---
	Positive	1.43	0.52	0.49 – 4.21	---	---	---
XR Cavities							
	None	BASELINE	---	---	BASELINE	---	---
	Present	0.45	0.03	0.22 – 0.94	0.50	0.07	0.23 – 1.06
Smoking							
	Never	BASELINE	---	---	---	---	---
	Previous	0.81	0.62	0.36 – 1.82	---	---	---
	Current	0.83	0.63	0.39 – 1.76	---	---	---

Table 4-3. *Logistic regression of baseline characteristics as exposure variables against an outcome of clinically significant (≥3xULN) peak ALT during treatment. Univariable odds ratios (crude) and multivariable odds ratios (adjusted) shown, with a univariable p value <0.10 as inclusion criteria for multivariable model. †Analysed as categorical variable*

4.2.3 Extreme Liver Enzyme Elevations

On standard therapy 7 of 639 (1.1%) patients exhibited a peak ALT result of ≥10xULN (range 10.1 – 19.1xULN), and among these patients the median time to peak ALT was 29 days of therapy (IQR 7-52). 6 of these patients were male, the median age was 33.3 years (IQR 28.5-40.9 years), and 2 patients were HIV positive. Alcohol use was confirmed as an underlying feature in 2 cases, and one of these patients had positive serology for acute hepatitis B. Most (5 of 7 patients) reported nausea or vomiting, and one reported abdominal pain within two weeks of the peak elevation.

5 Clinical Outcomes and Liver Enzyme Elevations on Standard TB Therapy

The precise impact of different levels of liver enzyme elevations on treatment outcomes has not previously been fully elucidated. In the previous chapter, there was an association between experiencing one or more clinically significant episodes of any

toxicity and an increased risk of failing to achieve microbiological cure. An awareness of the significance that should be attached to different levels of liver enzyme elevations would also inform monitoring practice and act as a motivating factor to drive policy changes that will improve patient care in a meaningful way.

The REMoxTB trial database contains information relating to early withdrawals, deaths and mycobacterial culture data up to 18 months after randomisation. The intention of this section was to investigate the number of patients who were withdrawn from standard TB therapy in the trial for liver-related reasons, and to use regression techniques to test the association between liver enzyme elevations and eventual microbiological cure.

5.1 Methods

"Liver-related withdrawals" were defined as patients withdrawn from treatment following a peak ALT or AST result ≥3xULN with no Adverse Events (either clinical or relating to biochemical tests) other than liver events recorded between their peak elevation and the time of withdrawal.

Patients were categorised according to their peak ALT value and an association with microbiological cure was assessed using logistic regression. Microbiological cure was defined as culture negative status at eighteen months (as in Section 4.1.1, Chapter 3). An ordered categorical variable was created for peak ALT in incremental steps of 1xULN, along with the binary exposure variables for clinically significant peak ALT (≥3xULN) and DILI, and these three exposure variables were independently regressed against the binary outcome variable for microbiological cure. Regression was controlled for age, sex, baseline weight, ethnicity, HIV status, and cavitation on chest X- ray.

5.2 Results

Only 11 patients were withdrawn from the standard therapy arm in the trial due to liver related events, and all of these patients survived to the end of the study. Their mean age was 37 years (±13.96 years) and 73% were male. The median ALT peak value in this group was 7.09xULN (IQR 2.83 – 10.05) occurring at 34.5 days, and the median peak AST was 5.99xULN (IQR 3.03 – 11.03) at 28.5 days. 4 of 11 (36.4%) patients had symptoms recorded; 4 with diarrhoea and/or vomiting and 3 also reported nausea. 10 of the 11 (90.9%) patients had achieved a microbiological cure at 18 months, after onward referral to the National Treatment Program. There was no culture data available after withdrawal for the final patient.

None of the exposure variables of peak ALT magnitude, clinically significant peak ALT, or DILI showed any statistically significant association with microbiological cure at 18 months (p value >0.10 for all three exposures, see Table 5-1). The 639 eligible patients taking standard therapy were included in the analysis. The model was controlled for age, gender, baseline weight, ethnicity, HIV status and cavitation on chest X-ray.

	Adj OR	95% CI	P value
Peak ALT Value	1.24	0.92 – 1.65	0.15
Age in years*	0.80	0.64 – 0.99	0.04
Female gender	1.06	0.57 – 1.97	0.87
Baseline Weight*	1.03	0.77 – 1.39	0.65
Ethnicity			
Black	BASELINE	---	---
Asian	0.49	0.25 – 0.94	0.03
Mixed Race	0.74	0.35 – 1.54	0.42
HIV-positive	0.78	0.25 – 2.42	0.67
Cavitation on CXR	0.85	0.43 – 1.70	0.65
Clinically Significant ALT Peak	1.55	0.45 – 5.38	0.487
Age in years*	0.79	0.64 – 0.99	0.04
Female gender	1.04	0.56 – 1.94	0.89
Baseline Weight*	1.03	0.77 – 1.39	0.83
Ethnicity			
Black	BASELINE	---	---
Asian	0.50	0.25 – 0.97	0.04
Mixed Race	0.72	0.34 – 1.50	0.38
HIV-positive	0.81	0.26 – 2.47	0.71
Cavitation on CXR	0.83	0.42 – 1.65	0.60
DILI	2.35	0.30 – 18.32	0.42
Age in years*	0.79	0.64 – 0.99	0.04
Female gender	1.05	0.56 – 1.95	0.88
Baseline Weight*	1.03	0.77 – 1.39	0.83
Ethnicity			
Black	BASELINE	---	---
Asian	0.50	0.26 – 0.97	0.04
Mixed Race	0.72	0.34 – 1.51	0.38
HIV-positive	0.79	0.26 – 2.44	0.69
Cavitation on CXR	0.83	0.42 – 1.65	0.59

Table 5-1. Odds ratio for achieving microbiological cure at 18 months after randomisation based on peak liver enzyme elevations during treatment in multivariable models. Peak ALT value is an ordered categorical variable grouping peak ALT results as increments of 1xULN, and all variables were investigated in a multivariable model controlling for age, gender, baseline weight, ethnicity, HIV status, and chest X-ray cavitation. *Age and baseline weight were categorised as presented in Table 4-1 of this chapter

6 Liver Enzyme Elevations in the Moxifloxacin-Containing Treatment Arms Compared to Standard TB Therapy

While they did not achieve non-inferiority, the more favourable toxicity profile demonstrated by the experimental arms in the previous chapter raises the question of whether they would still have a place in the management of TB if an optimal duration of treatment could be defined. Hepatotoxicity was the most prevalent form of toxicity in the experimental arms as well as in the standard TB therapy arm, but the magnitude of liver enzyme elevations and the incidence of DILI was not quantified in the previous chapter. A quantification of the differences seen between the standard TB therapy arm and the two experimental arms would help in understanding what, if any, role these regimens have in TB treatment. Whether they are potentially more suitable for use with patients who are more at risk of liver toxicity is of particular interest.

The main research questions to be addressed were determining the differences in timing and magnitude of liver enzyme elevations between the standard and experimental arms, and to identify any differences in the patient groups that experienced liver enzyme elevations on the different treatment arms.

6.1 Methods

6.1.1 Magnitude and Timing of Liver Enzyme Elevations

The peak liver enzyme values and timing in days since first dose of trial medication were calculated for all eligible patients on all three of the treatment arms, and the median value for ALT or AST calculated at each scheduled visit according to treatment arm. Depending on the number of patients in each group, the Chi squared or Fisher's exact test (if n ≤5 in any cell) was used to investigate the differences in peak ALT

values when grouped as ≥3xULN and <5xULN, ≥5xULN and <10xULN, and ≥10xULN. Comparisons made were pairwise comparing each experimental arm to standard TB therapy.

Kaplan-Meier curves were constructed for the time taken to reach the peak value in ALT and AST from first dose of medication for all patients in the trial. Kaplan-Meier curves were also constructed for the time taken to return to within the normal range from the peak value for patients with a peak ALT or AST value of >1xULN in all treatment arms. The logrank test was used to test for differences between the experimental and standard treatment arms for the events of interest (time to peak and time to normalise) occurring at any time point. Scatter plots were constructed to show the value and timing of elevations in ALT and AST in each treatment arm for patients with a peak value ≥3xULN.

6.1.2 Baseline Characteristics and Liver Enzyme Elevations

Baseline characteristics were tabulated for patients with peak ALT and/or AST ≥3xULN according to the treatment arm. The Chi square test was used to investigate for significant differences in the proportion of patients with clinically significant elevations according to baseline characteristics by treatment arm. Logistic regression was then used to explore the relationship between baseline characteristics for patients on each of the experimental arms and the binary outcomes of clinically significant peak ALT or DILI separately.

6.2 Results

6.2.1 Magnitude and Timing of Liver Enzyme Elevations

The median peak ALT result for all patients allocated to the isoniazid and ethambutol arms was 0.78 (IQR 0.53-1.23) and 0.73 (IQR 0.51-1.09), respectively. Figure 6-1

contains line graphs displaying the median value for the peak ALT and AST (as xULN) at scheduled visits during treatment, shown as weeks of treatment.

Patients receiving treatment on the isoniazid arm reached their peak ALT value at a median of 28 days (IQR 14-84), and at a median of 55 days (IQR 14-84) for those allocated to the ethambutol arm.

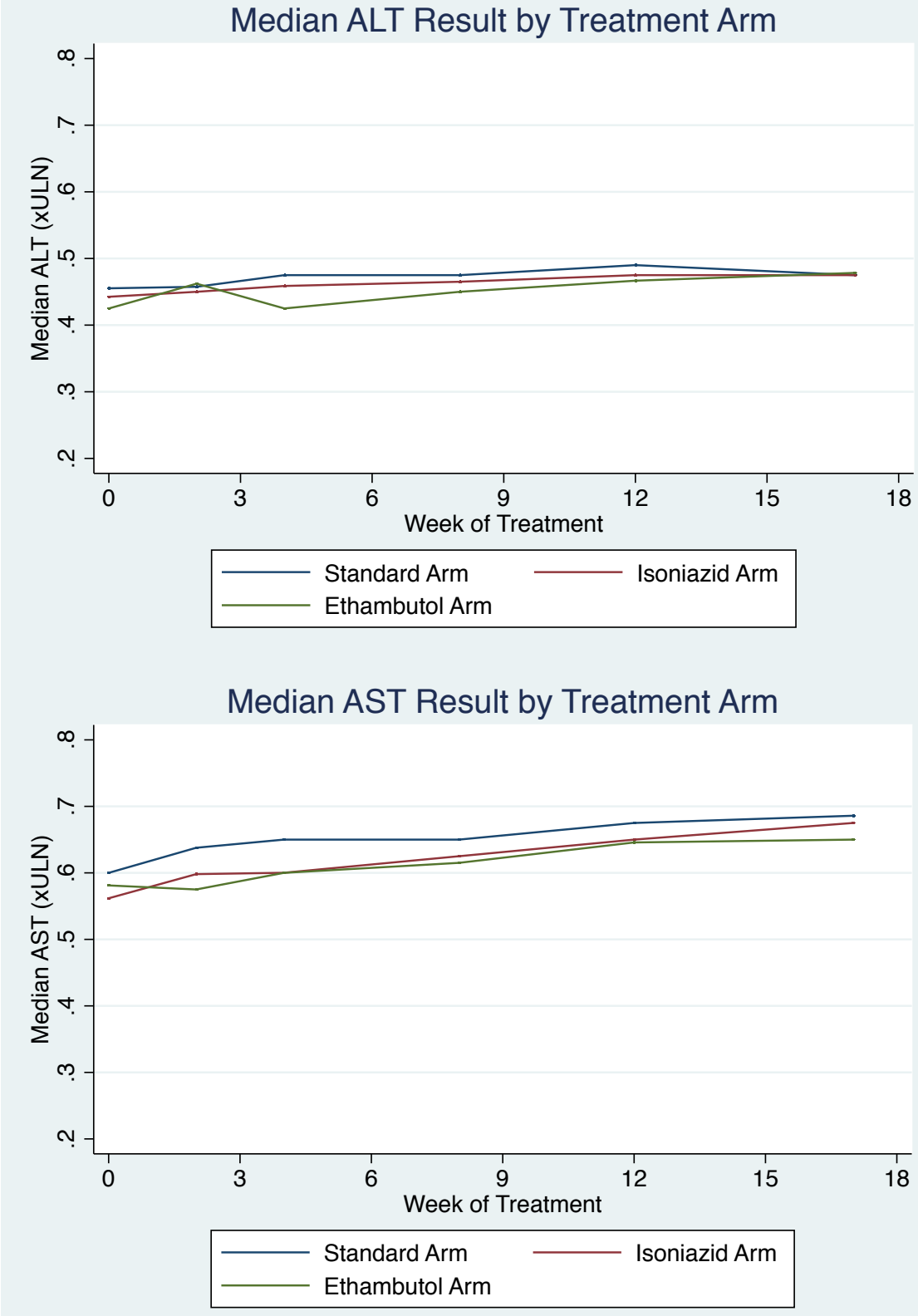

Figure 6-1. *Line graphs displaying the median peak ALT and AST value at scheduled visits for patients on all three treatment arms in the REMoxTB trial. ALT and AST both calculated as xULN*

Figure 6-2 contains Kaplan Meier curves for time to peak in all patients, and normalisation of elevated ALT and AST results for patients with peak result ≥1xULN.

The log rank test detected a significant difference (p value <0.001) for the time to peak ALT value between the standard (median time 28 days) and ethambutol (median time 55 days) arms with p value <0.001. The time for patients with peak ALT & AST values >1xULN to return to within the normal range from this peak value is also illustrated, with no significant difference detected between the treatment arms (p value >0.10).

There were 22 of 654 (3.4%) patients from the isoniazid arm who met the criteria for DILI. Out of these 22 patients, 5 patients reported diarrhoea and/or vomiting and 5 patients complained of nausea. The median RUCAM score for DILI patients was 10.5 (IQR 3.5 – 13). On the isoniazid arm 35 of 654 (5.4%) patients had recorded clinically significant peak ALT results.

14 of 634 (2.2%) patients on the ethambutol arm experienced liver enzyme elevations in keeping with DILI. From among the DILI patients, there were 2 patients who reported diarrhoea and/or vomiting, 1 patient who experienced nausea, and 2 patients who complained of abdominal pain. RUCAM scoring gave a median value of 7 (IQR 4.5 – 8.5) for those patients who experienced DILI. 25 of 634 (3.9%) patients on the treatment arm had peak ALT results that were clinically significant. Table 6-1 shows the frequency of peak ALT elevations and the timing for reaching a peak value and returning to within the normal range for patients on all three treatment arms.

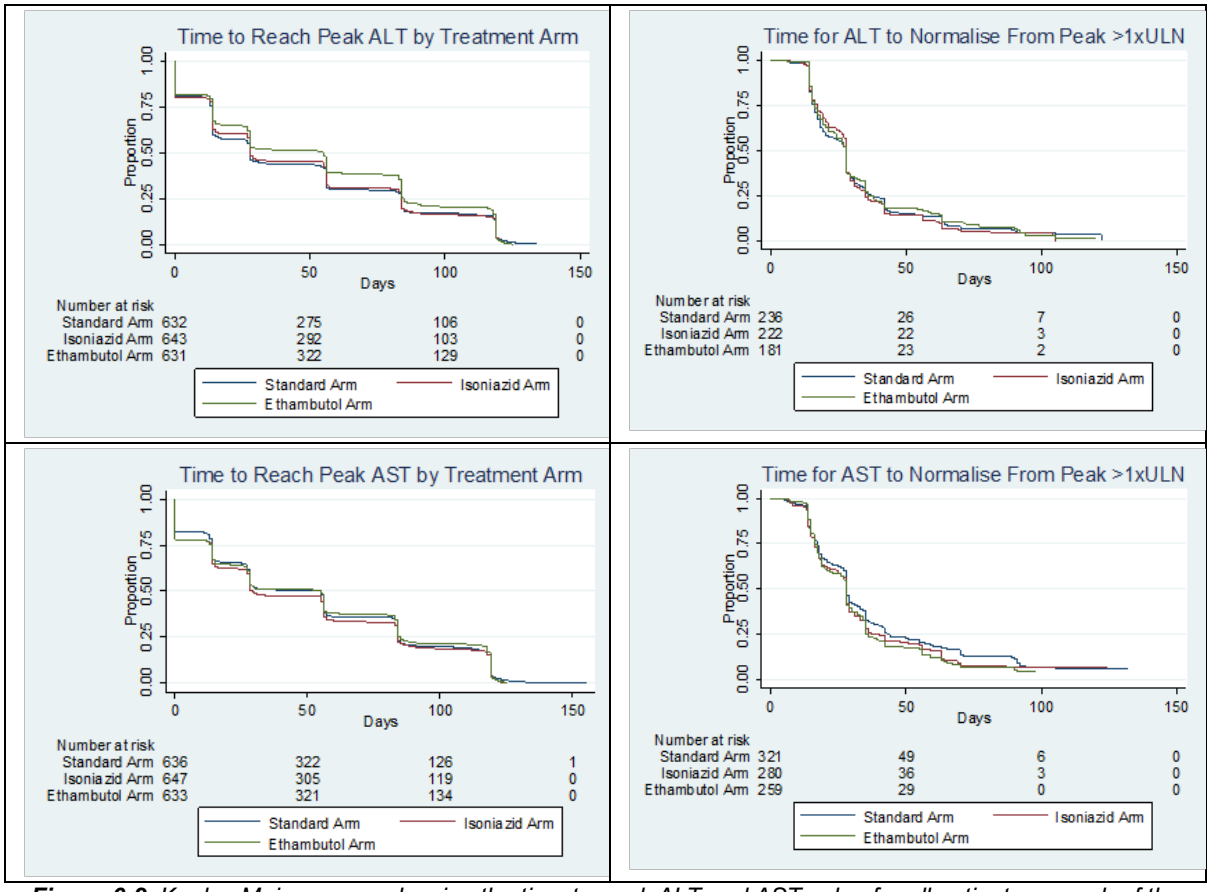

Figure 6-2. *Kaplan Meier curves showing the time to peak ALT and AST value for all patients on each of the treatment arms in the trial. The time to fall back into the normal range of ALT and AST is also shown for all patients with a peak ALT or AST result >1xULN*

The median time to reach peak ALT result was 23.5 days (IQR 14 – 55) for patients who developed DILI on the isoniazid arm. On the ethambutol arm, patients with DILI reached their peak ALT at 36.5 days (IQR 28 – 56). As shown in Table 6-1, the median time to peak ALT for clinically significant elevations was 18 days (IQR 14 – 84) and 55 days (14 – 84) on the isoniazid and ethambutol arms respectively. The scatter plots in Figure 6-3 illustrate the interquartile range for the magnitude of peak ALT and AST for patients with a peak value ≥3xULN on the three treatment arms, and also the interquartile range for the timing of the peak liver enzyme value. The wider interval for magnitude and timing of peak AST as compared to that for the peak ALT can be seen in all three treatment arms.

	Standard Arm (2EHRZ/4HR)	Isoniazid Arm (2MHRZ/2MHR)	Ethambutol Arm (2EMRZ/2MR)	p value
n[1]	634	649	634	N/A
Median peak ALT value as xULN (IQR)	0.83 (0.56-1.35)	0.78 (0.53-1.23)	0.73 (0.51-1.09)	0.046[5] <0.001[6]
Median time to peak ALT value in arm (days)	28 (14-84)	28 (14-84)	55 (14-84)	0.972[5] 0.017[6]
Median time to peak ALT (days) if ≥3xULN	28 (14-56)	18 (14-56)	28 (27-56)	0.755[5] 0.605[6]
Median time to ALT <1xULN[2] (days)	26 (15-42)	28 (19-42)	39 (30-61)	0.560[5] 0.270[6]
No. with peak ALT ≥3xULN & <5xULN(%n[3])	21 (3.3%)	17 (2.6%)	11 (1.7%)	0.204[7]
Bilirubin >2xULN	2 (0.3%)	4 (0.6%)	0 (0.0%)	0.142[7]
INR[4] >1.5	0 (0.0%)	3 (0.5%)	1 (0.2%)	0.098[7]
No. with peak ALT ≥5xULN & <10xULN (%n[3])	13 (2.0%)	16 (2.4%)	11 (1.7%)	0.656[7]
Bilirubin >2xULN	1 (0.2%)	2 (0.3%)	1 (0.2%)	0.792[7]
INR[4] >1.5	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A
No. with peak ALT ≥10xULN (%n[3])	7 (1.1%)	2 (0.3%)	3 (0.5%)	0.164[7]
Bilirubin >2xULN	2 (0.3%)	0 (0.0%)	1 (0.2%)	0.360[7]
INR[4] >1.5	0 (0.0%)	0 (0.0%)	0 (0.0%)	N/A
No of liver-related withdrawals	11 (1.7%)	7 (1.1%)	4 (0.6%)	0.178

Table 6-1. *Summary of the numbers of patients with significant elevations in liver enzyme concentration (≥3xULN, ≥5xULN, and ≥10xULN) by treatment arm. Median days from the start of treatment to reach individual patient peak concentrations and the number of patients withdrawing from treatment for liver-related reasons are reported. [1]Some patients not included because of missing ALT results, [2]If peak ALT ≥3xULN, [3]%n refers to percent of total patients in treatment arm, [4]Patients with known anticoagulant use were excluded, [5]Isoniazid arm against standard TB therapy, [6]Ethambutol arm against standard therapy, [7]Chi square (Fisher's exact if n<5)*

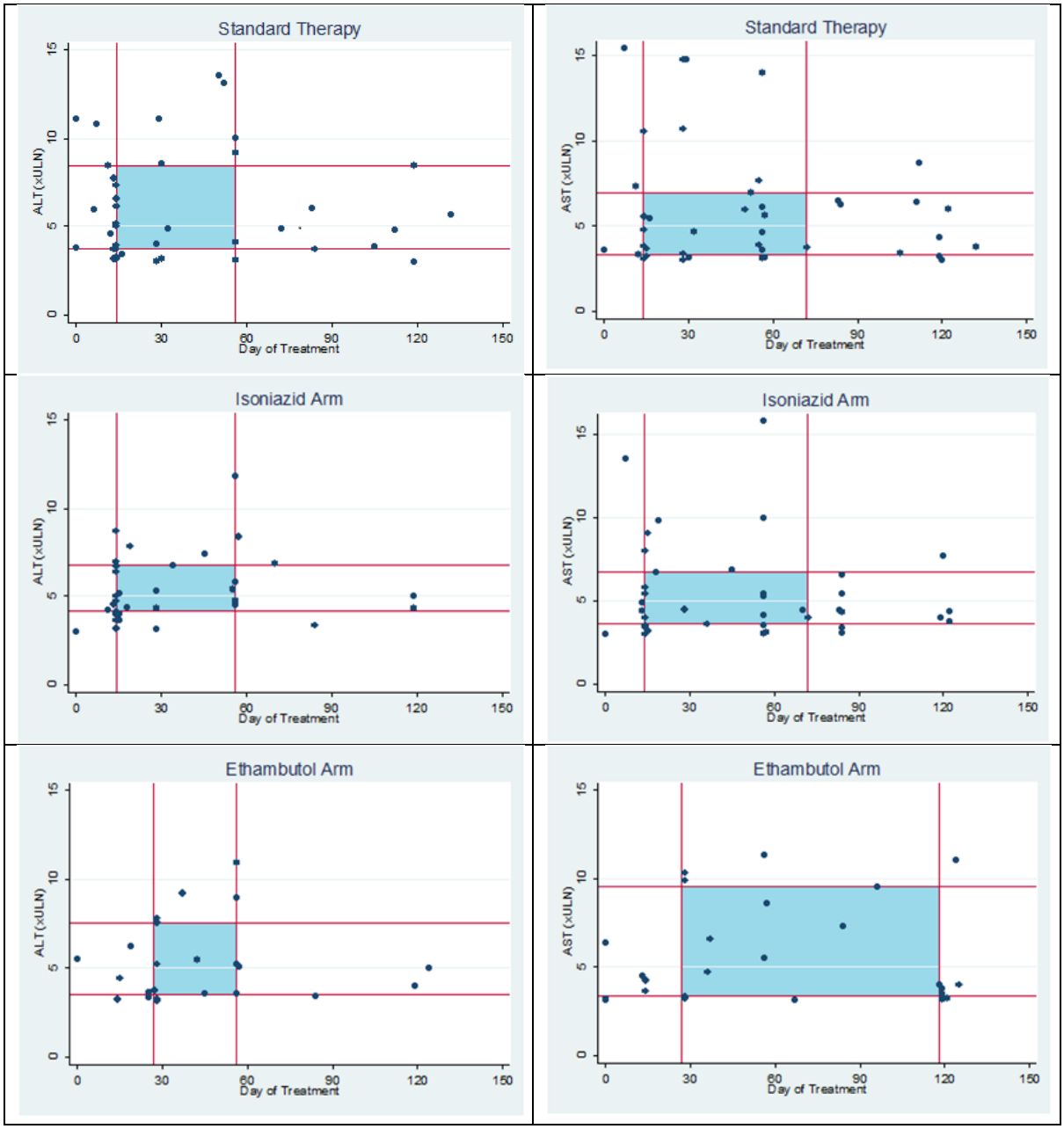

Figure 6-3. *Scatter Plots Illustrating Peak Values for ALT and AST in Patients When Peak Value ≥3xULN. The timing in days since first dose of treatment (x axis) and value of peak ALT & AST (y axis) is illustrated for each treatment arm for those patients with a peak value of ≥3xULN. The lines on the graphs indicate the interquartile ranges for the peak ALT/AST values and the timing of the peak values in this sub-group, with shaded area corresponding to the interquartile range for both time and elevation result*

6.2.2 Baseline Characteristics and Liver Enzyme Elevations

Table 6-2 reports the baseline characteristics for those patients on the experimental arms, and standard TB therapy for visual comparison, with a peak ALT and/or AST ≥3xULN. Significantly more Asian patients experienced ALT/AST ≥3xULN compared to other ethnic groups (11.4% (Asian) vs 6.7% (Black) vs 5.5% (Mixed), p value <0.001).

Applying the DILI criteria from Section 2.1.2, a significantly higher proportion of Asian patients in the trial experienced DILI (28 of 560, 4.8%) compared to the other ethnic groups (2.0% Black, 0% Caucasian, and 2.7% Mixed Race/Other [p=0.02]). Of the 67 Asian patients with a peak ALT/AST ≥3xULN, 57 of 67 (85.1%) were taking isoniazid-containing regimens and 10 of 67 (14.9%) were allocated to the ethambutol arm (p=0.008).

There were 2 patients in the isoniazid arm with a peak ALT ≥10xULN (39.6xULN with Bilirubin 1.7xULN & 11.9xULN at 17 & 56 days respectively). Both of them were male, HIV negative, and aged 21 and 28 years. No symptoms were recorded for either of these patients.

On the ethambutol arm, 3 patients developed peak ALT elevations of ≥10xULN (range 11.0 – 38.8xULN) in a range of 27-56 days. One of the three patients was male, the median age was 42.5 years (IQR 39.5-49.5 years), and one female patient who was HIV positive. This patient, for whom no predisposing conditions were identified suffered a severe and fatal course. Evidence of DILI arose after two weeks of treatment and she exhibited haemodynamic compromise, maculopapular rash, and symptoms of gastroenteritis. ALT peaked at 38.8xULN and total bilirubin 6.2xULN. No post-mortem was performed, with cause of death pronounced as “hepatitis of unknown cause”.

		Peak ALT or AST ≥3xULN[1]		
		Standard Arm (2EHRZ/4HR)	**Isoniazid Arm (2MHRZ/2MHR)**	**Ethambutol Arm (2EMRZ/2MR)**
n (%)		60 / 639 (9.4%)	52 / 654 (8.0%)	38 / 634 (6.0%)
Male Gender (%)		40 / 447 (9.0%)	38 / 449 (8.5%)	24 / 447 (5.4%)
Female Gender (%)		20 / 192 (10.4%)	14 / 205 (6.8%)	14 / 188 (7.5%)
Age in Years[2] (%)				
	18-24	13 / 186 (7.0%)	17 / 203 (8.4%)	11 / 172 (6.4%)
	25-34	21 / 184 (11.4%)	11 / 213 (5.2%)	9 / 210 (4.3%)
	35-44	16 / 142 (11.3%)	11 / 115 (9.6%)	8 / 122 (6.6%)
	45-54	2 / 88 (2.3%)	6 / 81 (7.4%)	8 / 86 (9.3%)
	≥55	8 / 38 (21.1%)	7 / 41 (17.1%)	2 / 44 (4.6%)
Baseline Weight in Kg (%)				
	<40	11 /63 (17.5%)	6 / 56 (10.7%)	5 / 56 (8.9%)
	40-49	22 / 218 (10.1%)	20 / 243 (8.2%)	11 / 224 (4.9%)
	50-59	16 / 242 (6.6%)	19 / 231 (8.2%)	12 / 236 (5.1%)
	60-69	8 / 88 (9.1%)	5 / 88 (5.7%)	8 / 86 (9.3%)
	≥70	3 / 28 (10.7%)	2 / 36 (5.6%)	2 / 33 (6.1%)
Ethnicity (%)				
	Black	20 / 295 (6.8%)	21 / 277 (7.6%)	17 / 290 (5.9%)
	Asian	34 / 194 (17.5%)	23 / 201 (11.4%)	10 / 193 (5.18%)
	Mix. Race	6 /149 (4.0%)	8 / 174 (4.6%)	11 / 151 (7.3%)
	Other	0 / 1 (0.0%)	0 / 2 (0.0%)	0 / 0 (0.0%)
HIV Pos (%)		7 / 46 (15.2%)	3 / 46 (6.5%)	4 / 48 (8.3%)
HIV Neg (%)		53 / 593 (8.9%)	49 / 608 (8.1%)	34 / 586 (5.8%)
Smoking History (%)				
	Never	31 / 298 (10.4%)	23 / 291 (7.9%)	16 / 279 (5.7%)
	Previous	11 / 155 (7.1%)	17 / 155 (11.0%)	9 / 166 (5.4%)
	Current	18 / 186 (9.7%)	12 / 208 (5.8%)	13 / 190 (6.8%)

Table 6-2. Relationship between elevation in liver enzyme concentration and patient characteristics at baseline reported by treatment regimen. Characteristics for patients across all treatment arms are shown according to peak ALT and/or AST result while taking treatment.

7 The Impact of Individual Drugs on Liver Enzyme Elevations

The effect on liver enzyme elevations from individual TB drugs is generally not well understood (with the notable exception of isoniazid (Nolan, Goldberg and Buskin, 1999; Jasmer *et al.*, 2002; Al-Darraji, Kamarulzaman and Altice, 2012)) owing to the fact that pulmonary TB is always treated with drugs in combination. “Liver-sparing”

regimens to treat TB are therefore normally the product of informed opinion guiding practice, with the exclusion of isoniazid and the inclusion of a fluoroquinolone a common practice; moxifloxacin has been shown to have a good safety profile overall, with a low risk of hepatotoxicity (Gosling *et al.*, 2003; Conde, Efron, Loredo, De, *et al.*, 2009; Dorman *et al.*, 2009).

A more detailed understanding of the hepatotoxic risk associated with individual drugs could allow for the creation of a selection of standardised "liver-sparing" regimens. This section utilised the design of REMoxTB to create three new treatment groups according to the presence or absence of isoniazid, moxifloxacin, and ethambutol in each of the trial treatment arms. The difference between these groups in terms of timing and magnitude of liver enzyme elevations was then described in an attempt to gain insight into the influence of these three drugs.

7.1 Methods

Patients were flagged as belonging to one of two "virtual" treatment arms from three different groups. This assignment was based on their treatment allocation in the trial to reflect the presence or absence of isoniazid, moxifloxacin and ethambutol. The three groups are shown in Table 7-1.

Drug of Interest	Virtual Treatment Arm	Study Regimens Included
ISONIAZID	Isoniazid-containing	Standard & Isoniazid Arm
	No-isoniazid	Ethambutol Arm
MOXIFLOXACIN	Moxifloxacin-containing	Isoniazid & Ethambutol Arm
	No-moxifloxacin	Standard Arm
ETHAMBUTOL	Ethambutol-containing	Standard & Ethambutol Arm
	No-ethambutol	Isoniazid Arm

Table 7-1. "Virtual" treatment arms used to investigate the impact of individual drugs on liver enzyme patterns. Patients were allocated to the "virtual" arm based on the regimen they received in REMoxTB according to the presence or absence of the three drugs of interest.

The median peak ALT value and time to reach the peak from first dose of medication was calculated for each of the "treatment arms" in the three groups. The values were compared using the Mann-Whitney U test to compare the mean time to peak value to

account for the skewed distribution of time to peak ALT & AST (histograms and inverse normal plots not shown). Box plots were created to display the data relating to time to peak ALT between “arms” in the groups. The number of patients experiencing DILI and clinically significant elevations was ascertained for each one of the “treatment arms” and a Chi square test applied to look for significant difference in the proportion of patients between the “arms”.

7.2 Results

Table 7-2 demonstrates that the proportion of patients with ALT and/or AST ≥3xULN was significantly higher in isoniazid containing arms (112 of 1,181, 8.7%) than the ethambutol arm (38 of 597, 6.0% [p value 0.039]). Similar percentages of patients had a peak ALT result of ≥5xULN: 3% in both the standard and isoniazid arms and 2% in the ethambutol arm. The results for the peak ALT/AST and timing of peak values for the groups divided according to the drugs present in the regimen can also be seen in Table 7-2. The patients receiving isoniazid as part of their treatment for TB reached their peak ALT at a median time of 28 days compared to 55 days in the no-isoniazid group (p value <0.01), and patients on the ethambutol arm reached a peak ALT result of ≥3xULN 9.5 days later than patients receiving the isoniazid-containing regimens combined (median timing 28 vs 18.5 days, p value 0.07).

There was a small but statistically significant difference for peak ALT value (0.78 & 0.73 vs 0.83xULN) and AST (0.93 & 0.90 vs 1.02xULN) in the moxifloxacin-containing arms when compared to the standard therapy group (p value <0.05). The median timing of the peak ALT was 29 days and 28 days in the moxifloxacin-containing and the no-moxifloxacin groups respectively (p value 0.137). While the median time to reach the peak AST in these two groups was 30 days and 50 days this was not found to be a significant difference with p value 0.351.

	ISONIAZID			MOXIFLOXACIN			ETHAMBUTOL		
Group	**INH**	**No-INH**	**P value***	**MXF**	**No-MXF**	**P value***	**ETH**	**No-ETH**	**P value***
Patients ALT/AST ≥3xULN	112/ 1,181 (8.7%)	38/ 597 (6.0%)	0.039	90/ 1,199 (7.0%)	60/ 579 (9.4%)	0.063	98/ 1,176 (7.7%)	52/ 602 (8.0%)	0.841
Med. Peak ALT Value (IQR)	0.80 (0.55-1.30)	0.73 (0.51-1.09)	0.001	0.75 (0.52-1.15)	0.83 (0.58-1.35)	0.001	0.78 (0.54-1.18)	0.78 (0.53-1.23)	0.942
Med. Time to Peak ALT (IQR)	28 (14-84)	55 (14-85)	0.005	29 (14-84)	28 (14-84)	0.137	28 (14-84)	28 (14-84)	0.196
Med. Peak AST Value (IQR)	0.95 (0.70-1.46)	0.90 (0.68-1.28)	0.002	0.91 (0.68-1.36)	1.02 (0.73-1.48)	<0.001	0.94 (0.70-1.38)	0.93 (0.67-1.45)	0.627
Med. Time to Peak AST (IQR)	30 (14-84)	54 (13-84)	0.728	30 (14-84)	50 (14-84)	0.351	52 (14-84)	28 (14-84)	0.203

Table 7-2. Peak ALT & AST values and timing presented in groups divided according to the presence of individual drugs. *Mann Whitney U test

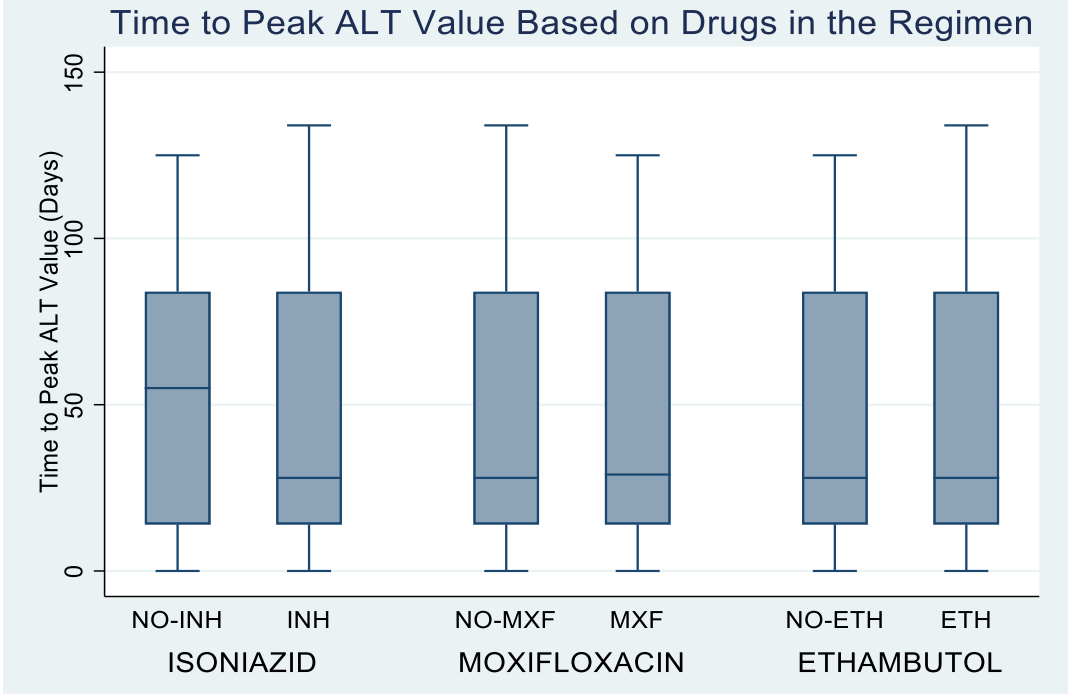

Figure 7-1. Boxplots demonstrating the time to reach a peak ALT and AST in groups based on the presence or absence of a specific drug in the treatment regimen

There were no significant differences detected using the Mann-Whitney U test in terms of magnitude or timing of peak ALT and AST results between the group receiving

ethambutol and the group that was not receiving it. The median values for the peak ALT/AST result and the time taken to reach a peak ALT were similar between the two groups. The median value for time to reach the peak AST was 52 days in the ethambutol-containing regimens but 28 days in the regimen with no ethambutol. However, the interquartile range was the same in both groups at 14-84 days, and the p value was not significant at 0.203. The median time to reach peak ALT according to the drugs present in the treatment regimen are visualised in the box plots of Figure 7-1.

8 Discussion

In this chapter it was demonstrated that almost 10% of patients taking standard TB therapy demonstrate ALT elevations ≥3xULN, however only a minority reported symptoms and approximately 3% of patients met the criteria for DILI. The majority of clinically significant elevations occurred within the first two months of standard TB therapy, and those patients whose peak ALT was greater than the upper limit of normal took approximately one month to return to within the normal range. Asian ethnicity was shown to be a significant risk factor for DILI associated with standard TB therapy, but female sex and HIV infection did not have a significant association with liver injury. DILI and clinically significant ALT elevations on treatment were not associated with worse treatment outcomes in this analysis. Of the three treatment arms, the ethambutol arm had the lowest incidence of DILI; the majority of clinically significant liver enzyme elevations occurred within the first two months of therapy on both experimental arms. There was a significant association with receiving isoniazid as part of a TB treatment regimen and increased risk of clinically significant liver enzyme elevations, and a shorter time to reach peak ALT elevation.

The data presented in this chapter provides a more definitive estimate of the incidence of TB therapy-related hepatic dysfunction due to the protocol-mandated sampling schedule and prospective collection of samples regardless of the clinical picture. The majority of published data has been retrospectively collected and the definition most frequently used for “hepatotoxicity” relates to patients who have developed symptoms and signs that have alerted a clinician to the need for blood tests to be performed (Fauzi *et al.*, 2004; Shang *et al.*, 2011; Chou *et al.*, 2014; Shin *et al.*, 2014; Yimer *et al.*, 2014; Bright-Thomas *et al.*, 2016; Chang *et al.*, 2018), and therefore the frequency of asymptomatic elevations and their significance in terms of management remained uncertain. This data would complement some of the largest datasets already published that quote rates of significant TB therapy-related hepatotoxicity of approximately 3-6% on standard TB therapy (Shang *et al.*, 2011; Shu *et al.*, 2013; Bright-Thomas *et al.*, 2016; Abbara *et al.*, 2017), as the proportion of patients seen to experience DILI on standard therapy was 3.4% in REMoxTB.

There has been an assumption that the majority of LBT elevations during TB treatment will occur within the first two to four weeks of therapy. The British and American Thoracic societies both recommend pre-treatment baseline blood tests followed by clinical assessment in all patients (Ormerod *et al.*, 1998; Saukkonen *et al.*, 2006). In patients deemed by the physician to be at higher risk of DILI or with existing liver disease or age >35 years old the ATS would recommend a further blood test between two to four weeks after initiating therapy (Saukkonen *et al.*, 2006). The BTS advises a policy of further weekly blood tests for two weeks and two weekly blood tests for the first two months for patients with known liver dysfunction (Ormerod *et al.*, 1998). Current guidance from the World Health Organisation does not include regular

monitoring for hepatotoxicity and instead symptoms would be the method of detecting liver dysfunction (World Health Organization, 2010).

In REMoxTB, the interquartile range for the time to reach a peak ALT ≥3xULN was 14-56 days for patients taking standard TB therapy, suggesting that 75% of these patients would have been detected by regular liver biochemical testing during the first 60 days of treatment. Based on the pattern of elevations observed, a monitoring schedule consisting of LBTs at baseline, 1 week (to identify early “elevators”), 2 weeks (lower end of the interquartile range), 4 weeks (median timing for elevations) and 8 weeks (upper end of interquartile range) would be possible, even in resource-poor settings. However, these findings suggest diminishing return for LBTs after 8 weeks of therapy in the absence of symptoms and, particularly in the absence of reliable data relating to treatment interruptions around enzyme elevations, it is not possible to make a definitive statement regarding the appropriate use of this suggested monitoring schedule in the field.

There are unanswered questions surrounding the significance of isolated ALT elevations: how best to evaluate these patients, and which are the most appropriate biochemical markers to be included in the evaluation of ALT elevations? (Saukkonen, Powell and Jereb, 2012) Isolated liver enzymes on treatment could reflect “hepatic adaptation” to the medication, wherein a transient liver biochemical test abnormalities resolve even with continued exposure to the drug and the patient remains clinically well throughout (Dara, Liu and Kaplowitz, 2016). One large cohort study of over 11,000 patients receiving isoniazid preventive therapy observed symptoms and signs of hepatotoxicity in only 0.15% of patients completing therapy (Nolan, Goldberg and Buskin, 1999), suggesting that the rates of DILI/enzyme elevations requiring intervention are actually very low with monotherapy and, by extension, with standard

TB therapy as well. Certainly, in this analysis a minority of patients with DILI or clinically significant elevations had recorded symptoms or bilirubin elevations and in the trial the majority of patients who experienced liver enzyme elevations went on to be cured of their disease.

A prospective series of 1223 patients with drug-induced liver failure demonstrated TB therapy-induced liver failure can often have a hyper-acute presentation and was associated with 67% mortality (Kumar *et al.*, 2010). The fatality seen on the ethambutol arm involving an ALT rise of 38xULN acts to highlight the risk of mortality for patients presenting with hyperbilirubinaemia and elevated liver enzymes. While the local physician evaluated this as “hepatitis of unknown cause”, it would seem reasonable to consider this as DILI. By comparison, the mortality associated with acute viral hepatitis is low and the majority of deaths are accounted for by the chronic sequealae of the disease (World Health Organization, 2017).

Despite most patients being clinically unaffected by ALT elevations on treatment, there was still an overall risk of 3.4% for DILI on standard TB therapy and higher rates of DILI among those patients with enzyme elevations at randomisation compared with the overall trial population. Given the challenging circumstances surrounding TB treatment (with much higher levels of HIV co-infection than seen in REMoxTB) we would still recommend the conservative monitoring schedule presented above to allow for the early detection of at risk patients. Given that the majority of patients with liver enzyme elevations were not withdrawn, there is some evidence in favour of attempting to re-introduce the same regimen following a treatment interruption as opposed to immediately changing to a more “liver-sparing” regimen that will potentially be less efficacious.

The application of the RUCAM scoring system to cases of DILI in REMoxTB showed a median score of 8 which indicates a “probable” causal relationship between standard TB therapy and liver enzyme elevations consistent with DILI. The RUCAM system for attributing causality for liver dysfunction to any medication being taken at the time was first published in 1993 (Benichou, Danan and Flahault, 1993; Danan and Benichou, 1993), and since that time has become an accepted tool for use in routine clinical practice, in research settings, and in support of regulatory decisions (Hayashi, 2016; Shahbaz, Mahajan and Lewis, 2017; Danan and Teschke, 2018). The main reservation in terms of using this score relate to the possibility of inter-observer variability and differing attributions of causality. However, in the original publication there was no disagreement in 84% of 50 DILI cases reviewed by four assessors (Danan and Benichou, 1993), and the RUCAM score has performed well in subsequent studies (Björnsson and Olsson, 2005; Danan and Teschke, 2015).

Increased risk of hepatotoxicity in certain ethnic groups has been variably demonstrated (Chamorro *et al.*, 2013; Du *et al.*, 2013; Lee *et al.*, 2014; Bright-Thomas *et al.*, 2016), and in this analysis there is evidence to support a higher risk of liver enzyme elevations in Asian patients. NAT2 is related to the metabolism of isoniazid, and polymorphisms affecting the cytochrome P450, CYP2E1, and NAT2 liver enzymes are associated with an increased risk of hepatotoxicity (Deng *et al.*, 2012; Chamorro *et al.*, 2013; Singla *et al.*, 2014). Certainly, the effect appeared to persist when patients were split into virtual treatment arms based on the presence or absence of isoniazid with the majority of Asian patients with a clinically significant peak ALT in the isoniazid-containing treatment arms. However, it needs to be remembered that there may be unmeasured factors unique to individual sites that confound the

relationship between ethnicity and liver function changes, such as alcohol use and viral hepatitis.

A higher proportion of older patients experienced enzyme elevations when taking standard TB therapy and this has been shown to be a risk factor for hepatotoxicity previously (Yee *et al.*, 2003; Shu *et al.*, 2013; Hosford *et al.*, 2014; Bright-Thomas *et al.*, 2016; Ortega-Alonso *et al.*, 2016). The proportion of patients in each age group with clinically significant peak ALT results was found to be statistically significant, but this effect was not seen in the regression analysis testing the relationship between patient age and DILI or clinically significant peak ALT. A probable explanation for this finding would lie in the small numbers in the highest age category that could create a false association, and therefore a tentative recommendation can be made to ensure that older patients are monitored more closely during treatment.

An association between significant hepatic events secondary to TB treatment and HIV positive status has been reported previously (Ungo *et al.*, 1998; Breen *et al.*, 2006; Saukkonen *et al.*, 2006; Hassen Ali *et al.*, 2013), however the precise magnitude of this risk is not clear. The observed higher rate of ALT/AST elevations in HIV positive individuals in this study is similar to other reports (Ungo *et al.*, 1998; Breen *et al.*, 2006; Hassen Ali *et al.*, 2013), although this did not achieve statistical significance in this analysis. Lower CD4+ counts have been significantly associated with risk for sub-clinical hepatotoxicity in HIV positive individuals on TB therapy (Yimer *et al.*, 2014). Exclusion of patients with CD4< 250cells/uL from the trial may have reduced the rates of hepatotoxicity. Also, none of the patients were enrolled on ART (as it was an exclusion criteria) and the vast majority did not receive ART during their TB treatment, reducing the impact of drug interactions on the incidence of toxicity.

Hepatotoxicity has previously been linked to a higher risk of poor treatment outcomes (Iseman, 2002; Shang *et al.*, 2011; WHO, 2017), often attributed to morbidity from liver dysfunction and the effect of treatment interruptions. There was a 92% rate of cure on the standard arm in REMoxTB (Gillespie *et al.*, 2014) and an interrogation of the outcome data in relation to liver enzyme elevations did not detect a significant association with higher peak ALT results, clinically significant peak ALT levels or DILI. This analysis used end points relating to microbiological cure (as treatment outcome) and withdrawal from treatment in the trial (as a surrogate for a more complicated course of treatment) to draw conclusions about the most appropriate response to liver enzyme elevations in clinical practice. The results presented give the clinician managing TB patients confirmation that, even in cases with clinically significant enzyme elevations or DILI, cure is still likely. However, an implication for policy from these findings is that patients participating in the trial were being closely monitored in the context of any liver enzyme elevations and this standard of care will likely have played a role in the outcome from treatment. The positive results seen among these patients should act as a strong motivator for policymakers to ensure that TB services are adequately funded to avoid unnecessarily unsatisfactory treatment outcomes which have implications at both the economic and public health level.

After observing unacceptably high rates of significant hepatotoxicity associated with the use of rifampicin and pyrazinamide to treat latent TB infection (Jasmer *et al.*, 2002; Stout *et al.*, 2003), a protective effect of isoniazid on the liver was hypothesised by some as a possible explanation (Lee *et al.*, 2002; Hest *et al.*, 2004) although drug dynamics in healthy individuals compared to unwell patients could also provide an explanation. In REMoxTB those patients taking isoniazid-containing regimens exhibit significantly higher and earlier peak ALT and AST values on treatment. Combined with

the significantly lower peak values seen in the moxifloxacin-containing arms (lowest in the ethambutol arm), this supports the use of moxifloxacin in liver-sparing regimens. Specifically, the low rate of enzyme elevations in the ethambutol arm could suggest a role for this as a liver sparing regimen but more work would be needed to investigate the ideal duration (Gillespie *et al.*, 2014).

8.1 Limitations

There are limitations to the work presented in this chapter. The patient population in the REMoxTB study was not fully representative of the diversity of tuberculosis patients, as CD4 and liver enzyme criteria were set for admission. Early in the REMoxTB study only AST was measured, and not ALT, which led to absent ALT results for some patients. The study protocol did not call for alkaline phosphate measurements and it was therefore not possible to apply Hy's Law criteria (Senior, 2006; Shen *et al.*, 2014) to clinically significant enzyme elevations (ALT >3xULN and Total Bilirubin >2xULN with normal alkanine phosphatase). The timings of temporary medication pauses were also not recorded with sufficient accuracy in the database, and there is also an inherent interval censoring in the data due to the scheduled sampling times that could reduce the precision of time-to-event analyses. Finally, the RUCAM scores were calculated by only one individual as part of this work, and a more robust method would have been to obtain scores from two separate individuals and ensure that there was sufficient agreement between them.

8.2 Conclusions

The work in this chapter constitutes one of the most detailed investigations of liver enzyme patterns during standard TB therapy available, based on prospective data collected according to prescribed schedule for all enrolled patients in the REMoxTB

study. Clinically significant ALT elevations predominantly occurred in the first two months of therapy, and a pragmatic monitoring schedule has been suggested that may increase the chances of identifying hepatotoxicity early. Older age (on standard therapy), Asian ethnicity and the presence of isoniazid in a treatment regimen have been shown as significantly associated with liver enzyme elevations during treatment. Closer monitoring of these patients during treatment may be beneficial. The analysis of the impact of individual drugs has provided reassurance for the inclusion of moxifloxacin in liver-sparing regimens and justification for the exclusion of isoniazid. Despite relatively high rates of liver enzyme elevations the majority of patients completed the course of their treatment with a high cure rate in the standard therapy, but questions still remain regarding the optimal approach to treatment interruptions. Standard TB therapy is lengthy and toxic, and this work further emphasises the need to develop a shorter, effective, and better-tolerated treatment to combat the ongoing global epidemic of tuberculosis.

Chapter Five: Toxicity and Adverse Events in HIV-positive, Anti-Retroviral Naïve Patients with High CD4+ Counts Treated for Pulmonary Tuberculosis

1 General Introduction

Infection with the human immunodeficiency virus (HIV) continues to act as a major driver of the global tuberculosis (TB) epidemic (Montales *et al.*, 2015; WHO, 2017), along with the development of drug resistance by the mycobacteria (Dheda *et al.*, 2017). There were an estimated 1.8 million new infections with HIV in 2016, and an estimated 36.7 million people living with HIV (PLHIV) in the same year (UNAIDS, 2017). The highest incidence (960 000) and prevalence (19.1 million) was seen in Eastern and Southern Africa (UNAIDS, 2017).

HIV infection results in greater chance of developing active TB by causing a widespread impairment of the body's immune system through an initial infection of CD4+ T helper cells, which then progresses to a reduced function of other T cell classes, B cells and dendritic cells (Pawlowski *et al.*, 2012; Maartens, Celum and Lewin, 2014; Bell and Noursadeghi, 2017). In keeping with this, the World Health

Organization (WHO) reported the African Region as having the highest proportion of HIV-positive cases in 2017, with 34% of reported TB patients that were tested for HIV demonstrating a positive result (WHO, 2017). However, it also needs to be remembered that the number of TB cases notified as having HIV co-infection only represents 46% of the estimated global burden of TB-HIV co-infection across the world according to the same WHO Global TB Report (WHO, 2017), and therefore the real scale of the problem is likely being underestimated in these figures.

The clinical presentation of TB-HIV co-infected patients has been reported as differing from that seen in HIV-negative patients with active TB disease and related to the degree of immunosuppression. HIV-positive patients have been shown as more likely to be sputum smear negative at presentation (Cattamanchi *et al.*, 2009; Kwan and Ernst, 2011; Gupta *et al.*, 2013), and either have fewer cavities or no cavitation seen on chest X-ray (Pitchenik 1985; Pedro-Botet et al. 1992; Post et al. 1995; Perlman et al. 1997). This is thought to reflect the frequently lower bacillary burden in co-infected individuals (Getahun *et al.*, 2007), translating into a delay in the diagnosis of TB in HIV-positive patients (Pablos Méndez & Frieden 1996; Palmieri et al. 2002), and consequently worse treatment outcomes (Githui et al. 1993; Banda et al. 2000; Hargreaves et al. 2001). There is also a higher risk of developing extra-pulmonary TB in patients infected with HIV (Sterling, Pham and Chaisson, 2010) and this can complicate the matter further.

There is an increased risk of toxicity in HIV-positive patients being treated for active TB noted in the existing literature (Yee *et al.*, 2003; Dworkin *et al.*, 2005; Lorent *et al.*, 2011; Tesfahuneygn, Medhin and Legesse, 2015), and as shown in the two previous chapters of the thesis. The reasons for increased toxicity are likely multifactorial relating to the chronic disease state brought about by HIV infection. HIV-positive

patients are known to have higher levels of resting inflammation (Sereti *et al.*, 2017; W. Yew *et al.*, 2018), and increased risk of cardiovascular, liver and kidney disease as a consequence of the infection (Triant, 2013; Cerrato *et al.*, 2015; Freiberg and So-Armah, 2015). Following from this primed state there is a higher risk of complications during standard TB therapy, especially hepatotoxicity (Saukkonen *et al.*, 2006; Yimer *et al.*, 2008; Lorent *et al.*, 2011). Additionally, approximately 15% of HIV-positive patients receiving ART will develop tuberculosis-immune reconstitution syndrome (TB-IRIS) with an attributed mortality of 1-3% (Lawn, Bekker and Miller, 2005; Burman *et al.*, 2007; Meintjes *et al.*, 2008). Rifampicin also induces the cytochrome enzymes in the liver and this can lead to reduced serum concentrations of ART, most notably the protease inhibitors (La Porte *et al.*, 2004; Maartens, Decloedt and Cohen, 2009), and there is the risk of overlapping toxicities (for example skin rashes and hepatotoxicity with nevirapine and efavirenz) (van Leth *et al.*, 2004; Shipton, Wester and Stock, 2009; Lehloenya and Dheda, 2012; Bonnet *et al.*, 2013). Unfortunately, the majority of the published work relating to HIV-TB infection is retrospective or observational in nature, often involves small numbers of patients, and uses variable definitions for toxicity (Blanc *et al.*, 2011; Maartens, Celum and Lewin, 2014; Mfinanga *et al.*, 2014; Pasipanodya and Gumbo, 2018; Walker *et al.*, 2018). This makes it difficult to reach an accurate estimate of the incidence of toxicity and the form it takes.

The impaired immune system in HIV-positive patients translates into a reduced ability of the host to clear the infection during the course of treatment (Shankar *et al.*, 2014; Bell and Noursadeghi, 2017), and consequently higher rates of treatment failure and relapse (or possibly re-infection) have been described in ART-naïve HIV-positive patients being treated for active TB (Zhou *et al.*, 2009; Getahun *et al.*, 2010; Ismail and Bulgiba, 2013). Furthermore, there are associations with a HIV-positive status and

increased rates of TB treatment default and loss to follow up; often related to the stigma associated with both infections, increased pill burden, and structural factors such as transport costs (Munro *et al.*, 2007; Gebremariam, Bjune and Frich, 2010; Legido-Quigley *et al.*, 2013; Tesfahuneygn, Medhin and Legesse, 2015). The overall risk of treatment failure is reduced in the context of adherence to ART (Manosuthi *et al.*, 2006; Sungkanuparph *et al.*, 2006; Zhou *et al.*, 2009), but despite significant gains in the roll-out of ART globally there are still many areas where treatment coverage is well-below 50% and there were an estimated 1.0 million AIDS-related deaths in 2016 (UNAIDS, 2017).

In Chapter 3 and 4 of this thesis, HIV-positive patients were identified as being at significantly higher risk of adverse events and toxicity during treatment with standard TB therapy, despite only patients with a CD4+ count of 250 cells/mm^3 or higher being eligible. In this chapter a matched sample of HIV-positive cases and HIV-negative controls selected from the REMoxTB database is compared using the detailed information collected for these patients over the course of the trial. The research questions proposed for the analysis of this matched sample were:

- What were the differences in the clinical characteristics of HIV-positive and HIV-negative patients with pulmonary TB at their presentation?
- How did the incidence of toxicity and change in measured physiological parameters compare between HIV-positive and HIV-negative patients in a sample matched for selected baseline characteristics?
- What differences were seen in terms of treatment outcomes between these ART-naïve, HIV-positive patients and the matched HIV-negative controls?

2 HIV-positive and HIV-negative Patients in REMoxTB

2.1.1 Baseline Characteristics for all Randomised Patients

In resource poor settings, where the availability of tests for HIV can be limited, clinicians managing TB in the community would benefit from a robust set of baseline characteristics that are clearly associated with TB-HIV co-infection. Patients presenting with these baseline characteristics would then made a priority for testing for HIV infection. Collaborative TB/HIV activities are listed as one of the components of Pillar 1 in the End TB Strategy (WHO, 2017), and targeting of those at greatest risk of co-infection could help the move towards universal HIV testing by introducing a more manageable, evidence-based goal in clinical settings that are already over-worked.

Almost 2000 patients were randomised onto one of the three treatment arms in REMoxTB and each one of them had detailed clinical and laboratory assessments carried out during the screening process. This section of the chapter aimed to use this data to compare the baseline characteristics for HIV-positive and HIV-negative patients across the trial prior to any treatment being administered to investigate for any characteristics that were significantly different between the two groups.

2.1.2 Matched Population of HIV-positive and HIV-negative Patients Assigned to Standard TB Therapy

One strategy to reduce unmeasured confounding in a non-randomised comparative study is to use a matched population. Cases that represent the disease-group or intervention of interest are assigned one or more controls who do not have the disease or did not receive an intervention. These controls will be similar to the cases in terms

of gender, age, ethnicity, and any other variables thought to be relevant. The intention is to reduce inherent variability between the case and control groups, and allow for a more unbiased estimation of disease impact or treatment effect.

In this section, coarsened exact matching was used to take advantage of the detailed clinical information available for HIV positive and negative patients assigned to standard TB therapy in REMoxTB. This technique was employed to create a matched case-control sample for analysis in the chapter to attempt to reduce bias when estimating the impact of HIV infection on treatment toxicity and outcomes.

2.2 Methods

2.2.1 Baseline Characteristics for all Randomised Patients

The baseline characteristics for all randomised patients across the three treatment arms in REMoxTB were compared to determine any significant differences. Patients were categorised according to their randomisation status and HIV status in the database, and patients who were not randomised were excluded from the analysis. Characteristics from before the initiation of treatment were collected for HIV-positive and HIV-negative patients, and presented in table format for visual inspection and comparison of proportions in each of the two patient categories. The Chi square test was applied to investigate for statistically significant differences in the proportion of patients with each of the characteristics at presentation accounting for HIV-status.

2.2.2 Matched Population of HIV-positive and HIV-negative Patients Assigned to Standard TB Therapy

Coarsened exact matching (CEM) was used to create a population of HIV positive cases matched to HIV negative controls. This is a monotonic imbalance-reducing matching method, wherein the variables to match the population were chosen prior to

the matching process based on clinical judgement regarding their probable relationship to the patients' response to treatment over time and eventual treatment outcome. The method was developed by Iacus et al (Iacus, King and Porro, 2012) and implemented in a user written command for Stata (Newton *et al.*, 2009). The variables for age, gender, ethnicity and trial site were selected as the matching variables for HIV-positive and HIV-negative patients allocated to the standard TB therapy arm in REMoxTB with the matched sample drawn from all patients randomised to this treatment arm in the trial. These were considered the most relevant clinical variables, and attempting to match on additional variables caused up to 60% of the HIV-positive patients being lost from the analysis population.

CEM converted the selected variables into bins based on an automatic algorithm before matching HIV-positive and HIV-negative patients into strata with weights provided for each patient within the strata. A copy of the selected variables was made and coarsened according to the binning algorithm contained within the CEM program before one stratum was created for each unique observation of the copied dataset. Each observation was then placed into a stratum, and these strata were assigned to the original dataset. Strata that did not contain at least one HIV-positive and HIV-negative patient were then dropped, and this final matched sample of HIV-positive and HIV-negative patients receiving standard TB therapy was adopted as the sample for analysis in the remainder of the chapter. The advantage of this process of CEM over one-to-one matching is that more patients can be matched using pre-defined coarsened variables with a specified maximum bound on the imbalance on the moments of each matching variable. The resulting analysis population is larger than that obtained from matching on the basis of exact values.

The test for global imbalance in the matching variables was based on the difference between the multidimensional histogram of the variables in the HIV-positive and HIV-negative patients, and presented as the L_1 statistic (Iacus, King and Porro, 2012). The covariates were initially coarsened into bins, and then the discretised variables cross-tabulated for the case and control groups separately. The relative frequencies for the case and control units were recorded and the measure of imbalance given by the absolute difference over all the cell values. The L_1 statistic indicates the measure of global balance between perfect global balance ($L_1 = 0$) and complete separation between the matching variables ($L_1 = 1$).

Baseline characteristics, beyond those used to match the patients, were collected for the HIV-positive and HIV-negative patients in the matched sample on standard TB therapy. The maximum number of matching variables was chosen that allowed the greatest number of HIV-positive patients to be matched to HIV-negative controls. However, to ensure that there were no significant differences in the matched sample as a result, characteristics were tabulated to demonstrate the proportions according to HIV status. Statistical differences in the sample proportions for characteristics in HIV-positive and HIV-negative patients were sought using the Chi square test. Patients were excluded from the trial if they were taking ART at screening; however files in the dataset relating to concomitant medications were used to determine the number of patients started on ART during the trial and the timing of initiation. It should also be noted that over 200 HIV-positive patients were excluded at screening due to either CD4+ counts that were less than 250 cells/mm^3 at baseline or because of ART use (see CONSORT diagram in REMoxTB New England Journal paper, Appendix One).

2.3 Results

2.3.1 Baseline Characteristics for all Randomised Patients

There were 37 of 140 (26.4%) HIV-positive patients and 349 of 1790 (19.5%) HIV-negative patients with no cavitation seen on chest X-ray (Chi square p value 0.02). However, the proportion of patients with a TTP less than the median value for the total analysis sample was similar irrespective of HIV status: 73 of 140 (52.1%) of HIV-positive and 841 of 1790 (47.0%) HIV-negative patient sputum samples became positive faster than the total sample median time at baseline (p value 0.24).

There were 128 of 140 (92.1%) HIV-positive patients classed as “black” ethnicity and 734 of 1790 (41.0%) HIV-negative patients; all of the black HIV-positive patients were enrolled at South African sites (see Table 2-1). Only 3 of 140 (2.2%) HIV-positive patients were Asian ethnicity, compared to 587 of 1790 (32.8%) HIV-negative patients belonging to this ethnic group. A higher proportion of randomised HIV-positive patients were female, 62 of 140 (44.3%), compared to 523 of 1790 (29.2%) of HIV-negative patients (p value <0.001). The median CD4+ count was 399 cells per mm^3 (IQR 316 – 526) among the HIV-positive patients.

		HIV-positive	HIV-negative
n		140	1790
Gender			
	Male	78 (55.7%)	1267 (70.8%)
	Female	62 (44.3%)	523 (29.2%)
Age in Years			
	<25	20 (14.3%)	545 (30.5%)
	25 – 35	60 (42.9%)	546 (30.5%)
	>35	60 (42.9%)	699 (39.0%)
Baseline Weight			
	<40	4 (2.9%)	171 (9.6%)
	40 – 45	15 (10.7%)	311 (17.4%)
	>45 – 55	50 (35.7%)	716 (40.0%)
	>55 – 75	65 (46.4%)	552 (30.8%)
	>75	6 (4.3%)	40 (2.2%)
Ethnicity			
	Black	128 (92.1%)	734 (41.0%)
	Asian	3 (2.2%)	587 (32.8%)
	Caucasian	0 (0.0%)	3 (0.2%)
	Other	8 (5.8%)	8 (5.8%)
Smoking Status			
	Never	70 (50.0%)	800 (44.7%)
	Previous	38 (27.1%)	438 (24.5%)
	Current	32 (22.9%)	552 (30.8%)
Chest X-ray			
	Cavities	80 (57.1%)	1247 (69.7%)
	No cavities	37 (26.4%)	349 (19.5%)
MGIT TTP			
	≥ Median	67 (47.9%)	949 (53.0%)
	< Median	73 (52.1%)	841 (47.0%)

Table 2-1. Baseline characteristics of all randomised patients for REMoxTB according to HIV status. Column percentages included in cells for proportion of total n in for HIV-positive and HIV-negative patients belonging to categories of baseline characteristic

2.3.2 Matched Population of HIV-positive and HIV-negative Patients Assigned to Standard TB Therapy

Coarsened exact matching (CEM) produced a matched population of 42 HIV-positive cases to 220 HIV-negative controls all receiving standard TB therapy in REMoxTB.

The patients were allocated to 18 matching strata leaving 4 HIV-positive patients and 374 HIV-negative patients un-matched due to an inability to fit them into one of the strata. The multivariate L_1 statistic was 0.152 which indicated overall low global imbalance according to the matching variables and gives reassurance that the matching process worked. The univariate L_1 statistic for imbalance across the matched population was 0.077 for age, and less than $3x10^{-16}$ for sex, ethnicity, and trial site.

There were no significant differences identified between the HIV-positive and HIV-negative patients in the matched sample when the Chi square test was applied to the proportions in the extended list of baseline characteristics. The median CD4+ cell count among the HIV-positive patients was 370 cells per mm^3 (IQR 307 – 456). The proportion of patients with cavitation seen on chest X-ray at baseline was lower for HIV positive patients, as was seen for all patients in the trial, but this fell short of reaching statistical significance (p value 0.06). As was noted when all randomised patients were compared, the proportion of patients with a TTP less than the median sample time was similar with 20 of 42 (47.6%) HIV-positive and 102 of 220 (46.4%) HIV-negative patients falling into this category. The process of CEM addressed the previous large difference in ethnicity for HIV-positive and HIV-negative patients, and Table 2-2 demonstrates similar proportions of patients in each ethnic group (p value 0.58). Only 10 HIV-positive patients were started on ART following randomisation into the trial at a median time of 180 days after first dose of trial medication.

		HIV-positive	HIV-negative
n		42	220
Gender			
	Male	23 (54.8%)	154 (70.0%)
	Female	19 (45.2%)	66 (30.0%)
Age in Years			
	<25	4 (9.5%)	40 (18.2%)
	25 – 35	17 (40.5%)	84 (38.2%)
	>35	21 (50.0%)	96 (43.6%)
Baseline Weight			
	<40	2 (4.8%)	9 (4.1%)
	40 – 45	7 (16.7%)	39 (17.7%)
	>45 – 55	16 (38.1%)	83 (37.7%)
	>55 – 75	15 (35.7%)	83 (37.7%)
	>75	2 (4.8%)	6 (2.7%)
Ethnicity			
	Black	38 (90.5%)	186 (84.6%)
	Asian	2 (4.8%)	20 (9.1%)
	Other	2 (4.8%)	14 (6.4%)
Smoking Status			
	Never	18 (42.9%)	114 (51.8%)
	Previous	11 (26.2%)	61 (27.7%)
	Current	13 (31.0%)	45 (20.5%)
Chest X-ray			
	Cavities	26 (61.9%)	160 (72.7%)
	No cavities	11 (26.2%)	32 (14.5%)
MGIT TTP			
	≥ Median	22 (52.4%)	118 (53.6%)
	< Median	20 (47.6%)	102 (46.4%)

Table 2-2. Baseline characteristics of matched patients in analysis sample from REMoxTB according to HIV status. Column percentages included in cells for proportion of total n in for HIV-positive and HIV-negative patients belonging to categories of baseline characteristic

3 Toxicity and Adverse Events in the Matched Population Assigned to Standard TB Therapy

Previous chapters have demonstrated that toxicity can take many different forms in the context of TB chemotherapy, and the approach to monitoring and management will vary depending on this. An awareness of the population-level risk and how this changes according to a patient's characteristics is invaluable to the frontline clinician treating these patients. This information allows physicians to quickly identify those patients who are most likely to have a more complicated treatment period (with the associated negative impact on treatment outcomes demonstrated in earlier chapters), and ensure that they are more closely monitored and more proactively supported during their treatment.

It has been demonstrated that HIV positive patients were at higher risk of toxicity in REMoxTB. Section 2 of this chapter investigated the baseline characteristics of ART-naïve TB-HIV-co-infected patients with high CD4+ counts and generated a matched cohort of cases and controls, and this section aimed to investigate what form toxicity takes in these HIV-positive patients receiving standard TB therapy and when it is most likely to occur in comparison to the matched controls.

3.1 Methods

The number of HIV-positive and HIV-negative patients who experienced ≥1 grade 3 or 4 adverse event (AE) was calculated for both the total grade 3 or 4 AEs and only for those that were considered related by the reporting clinician. Grade 3 or 4 AEs with and without recorded start dates were included when identifying these patients and events were collated according to System Organ Class (SOC) and Preferred Term (PT) for HIV-positive and HIV-negative patients.

The median time to the first grade 3 or 4 AE was calculated according to the patient's HIV status for all grade 3 or 4 AEs and for those that were considered related-only. Kaplan-Meier curves were constructed using the time to first grade 3 or 4 AE among the HIV-positive and HIV-negative patients by calculating the difference between the onset date of the event and the date of first dose of standard TB therapy; the earliest grade 3 or 4 AE was considered the failure event with censoring at the date of last review for those patients who did not experience a grade 3 or 4 AE. Finally, a Cox proportional hazards model was used to test the association between HIV status and the incidence of first (total and related-only) grade 3 or 4 AE.

3.2 Results

A total of 20 of the 42 (47.6%) HIV-positive patients experienced ≥1 grade 3 or 4 AE, as opposed to 34 of the 220 (15.5%) HIV-negative patients. One or more grade 3 or 4 AEs considered to be at least possibly related to treatment was reported by 11 of 42 (26.2%) HIV positive patients and 14 of 220 (6.4%) HIV-negative patients. There were 37 of 42 (88.1%) and 72 of 220 (32.7%) patients who reported one or more grade 1 or 2 AE from among the HIV-positive and HIV-negative group, respectively. These proportions changed to 25 of 42 (59.5%) and 52 of 220 (23.6%) patients in the HIV-positive and HIV-negative groups when only related grade 1 or 2 AEs were included.

Hepatobiliary disorders accounted for the majority of the total grade 3 or 4 AEs for both HIV-positive and HIV-negative patients in the matched population (see Table 3-1). Respiratory and thoracic disorders, blood and lymphatic disorders, and infections and infestations were among the five most common SOCs for both HIV-positive and HIV-negative patients.

	Adverse Event SOC	Frequency	Percent
HIV Positive	**Hepatobiliary disorders**	12	30.00
	Respiratory and thoracic disorders	7	17.50
	Blood and lymphatic disorders	6	15.00
	Infections and infestations	3	7.50
	Metabolism and nutrition disorders	3	7.50
HIV Negative	**Hepatobiliary disorders**	15	25.00
	Blood and lymphatic disorders	13	21.67
	Respiratory and thoracic disorders	10	16.67
	Infections and infestations	5	8.33
	Gastrointestinal disorders	4	6.67

Table 3-1. System Organ Classes for the five most frequently reported grade 3 or 4 Adverse Events (AEs) reported by HIV-positive and HIV-negative patients in the matched population during enrolment in the REMoxTB trial. Percentage of total number of grade 3 or 4 AEs provided, but not all events are shown

"Hepatic enzyme increased" was the most common related grade 3 or 4 adverse event PT for both the HIV-positive patients (3 of 18 PTs, 16.7%) and the HIV-negative (6 of 24 PTs, 25.0%). The 5 most common PTs from related and unrelated grade 3 or 4 AEs reported for the HIV-positive and HIV-negative patients are shown in Table 3-2. "Hepatic enzyme increased" was the most commonly reported PT for HIV-negative patients, and the five most common PTs for HIV-positive patients all had the same reporting frequency.

	Preferred Term	Frequency	Percentage
HIV Positive	**Hepatic enzyme increased**	3	7.50
	GGT increased	3	7.50
	AST increased	3	7.50
	Hyponatraemia	3	7.50
	Respiratory disorder	3	7.50
HIV Negative	**Hepatic enzyme increased**	8	13.33
	Haemoglobin decreased	4	6.67
	GGT increased	3	5.00
	Haemoptysis	3	5.00
	Prothrombin Time prolonged	3	5.00

Table 3-2. The five most common Preferred Terms used for grade 3 or 4 adverse events reported by HIV-positive and HIV-negative patients in the REMoxTB trial. Adverse events considered to be related and those classed as unrelated are included, and the percentage of all Preferred Terms accounted for by each entry is provided although not all Terms are listed

The hazard ratio for experiencing a grade 3 or 4 AE for HIV-positive patients was significantly elevated compared to HIV-negative patients at 3.25 (95% CI 1.87 – 5.66, p value <0.01). The hazard ratio remained significant for HIV-positive patients when only related grade 3 or 4 AEs were included (HR 3.77, 95% CI 1.65 – 8.60, p value <0.01). The median time to first grade 3 or 4 AE was 54 days (IQR 15.5 – 59.0) for HIV positive patients and 29.5 (IQR 9 – 119) for HIV negative patients in the analysis sample. When only related events were included the time to first grade 3 or 4 AE was 56 days (IQR 29 – 57) for HIV-positive patients and 50 days (IQR 19 – 56) for HIV-negative patients. Figure 3-1 presents Kaplan-Meier curves for the time to first grade 3 or 4 AE according to the patient's HIV status.

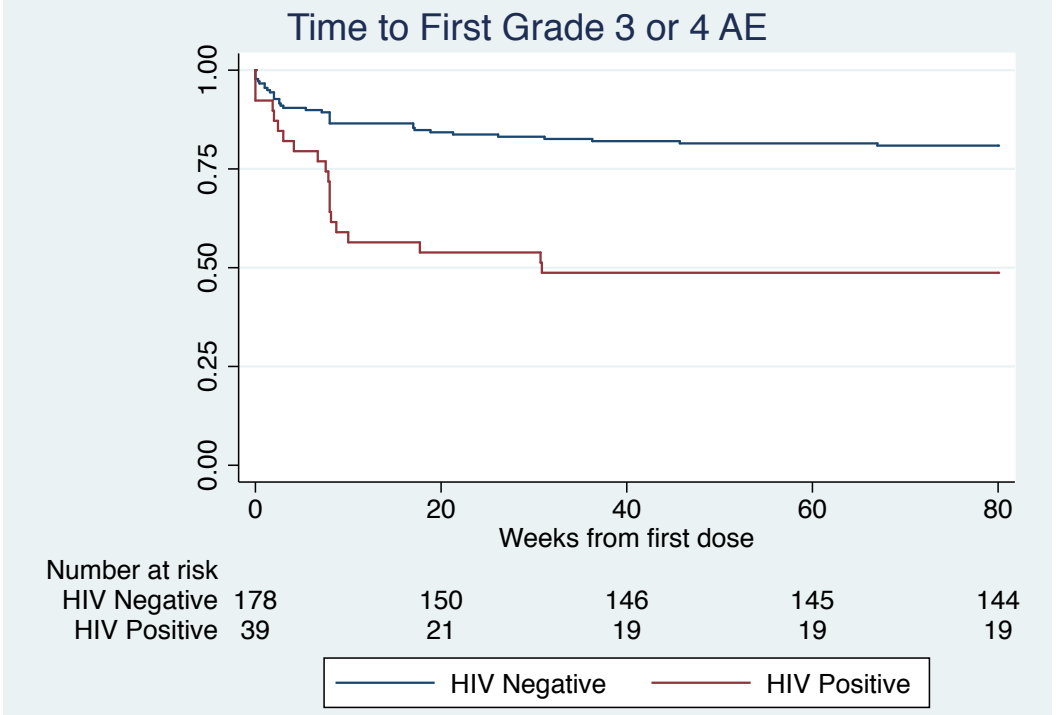

Figure 3-1. Kaplan-Meier curves demonstrating the time to first related or unrelated grade 3 or 4 adverse event according to patient HIV status in the matched sample

4 Longitudinal Analysis of the Matched Population Assigned to Standard TB therapy

Knowledge of the expected clinical course a patient follows during treatment for a disease allows the treating clinician to make informed judgements regarding their care. This relates closely to the issue of toxicity during treatment, as a deviation from the expected course during therapy for a disease will prompt the treating physician to consider a change in therapy. In the case of TB, a change in therapy will generally translate into the use of less effective drugs and a longer duration of treatment. Patients with HIV infection suffer from a chronic disease and therefore will be less well, compared to HIV-negative patients with TB, but this makes an accurate quantification of measured parameters even more important as there is a risk that warning signs will

be inappropriately dismissed or that expected findings will be over-interpreted and treatment unnecessarily changed.

In this fourth section of the chapter an attempt is made to illustrate the patient journey on standard TB therapy for largely ART-naïve HIV positive patients and how this compares to a group of matched HIV negative controls. Specifically, involving the identification of which clinical and laboratory parameters are significantly different at the start and end of therapy between HIV-positive and HIV-negative patients.

4.1 Methods

The mean values were calculated for haemoglobin result, patient weight, number of TB symptoms reported and alanine transferase (ALT) result at each protocol-scheduled visit for HIV-positive and HIV-negative patients in the matched population. Blood samples were collected for haemoglobin at weeks 0, 2, 8, 12, and 17 routinely for all patients. Weight and number of TB symptoms reported was documented for each protocol-scheduled visit during enrolment in the trial. Serum ALT was measured at weeks 0, 2, 4, 8, 12, and 17 for all patients taking trial medication. The mean value for each variable was calculated for each scheduled visit when the data was collected for the HIV-positive and HIV-negative patient samples, and measurements taken at unscheduled visits were discarded. Four different longitudinal plots were created with the mean variable value plotted on the y axis and the number of weeks from the first dose of standard TB therapy on the x axis.

Generalised estimation equations (GEE) were used to investigate the association between HIV status and the longitudinal change in haemoglobin, weight, symptom count, and ALT results at protocol-scheduled visits. GEEs were fit using the longitudinal variable of interest as the dependent variable with Gaussian distribution,

an identifier link between dependent variable and predictor, and exchangeable correlation matrix. The latter rejects the assumption of independence between pairs of time points and instead constrains the correlations between values at paired time points to be equal. Weeks after first dose of treatment, a multiple of week number and HIV flag to test interaction, and the week number squared were also included as predictors in the GEE.

The first and last haemoglobin, weight, TB symptom count, and ALT results for each patient in the matched population were used as dependent variables in a multivariate regression model with HIV status as the independent variable. Zellner's seemingly unrelated regression was used to build a multivariate model testing the effect of HIV status on the variables of interest, chosen to allow for correlation between the error terms in the individual regression equations using feasible generalised least squares. To allow for a more appropriate comparison of variables in the model, the screening value and the result at week 17 were used as all variables had these time points routinely measured.

4.2 Results

Patients with HIV infection demonstrated a lower mean haemoglobin result at baseline and at four months of treatment with standard TB therapy compared to HIV-negative patients. The mean haemoglobin at the beginning of treatment was 0.89xLLN and 1.00xLLN at week 17 in patients with HIV infection, and 0.93xLLN and 1.08xLLN at the same time points for HIV-negative patients. The mean haemoglobin at scheduled visits according to HIV status is shown in Figure 4-1. There was a significant effect of HIV status on the mean haemoglobin over the first 17 weeks of treatment using GEE with coefficient -0.05 (95% CI [-0.098] – [-0.003], p value 0.04).

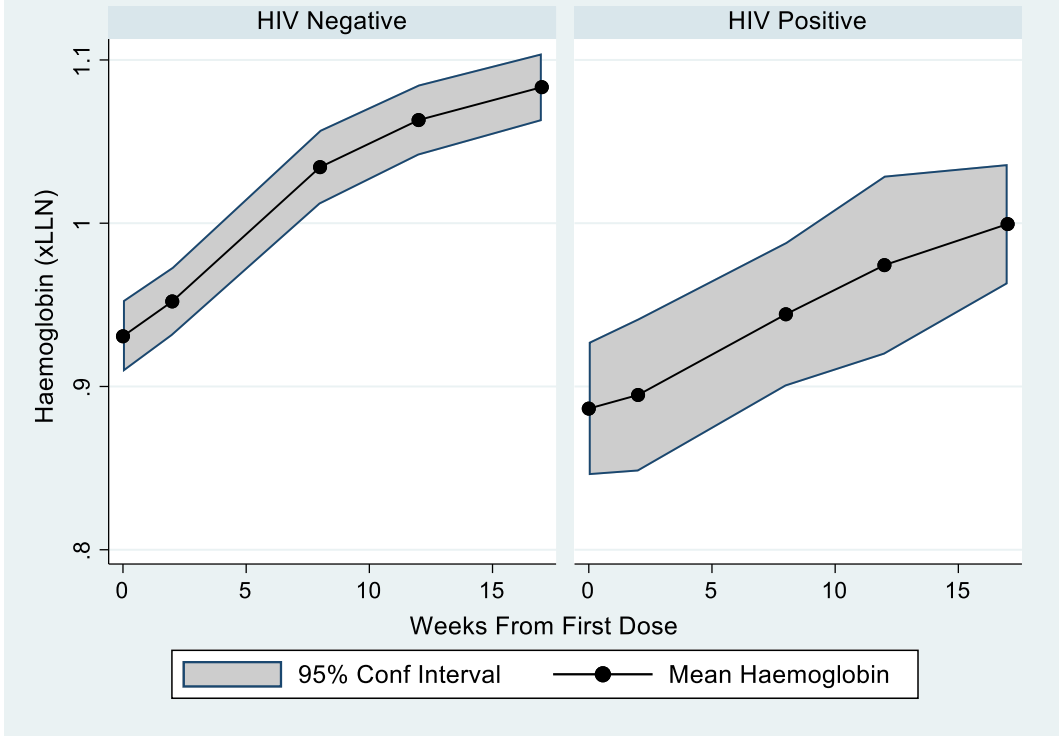

Figure 4-1. Longitudinal plots to present the mean value of haemoglobin in HIV-positive and HIV-negative patients taken at protocol scheduled visits in REMoxTB. Test results taken at unscheduled visits are not included in this graph, and haemoglobin is presented as a multiple of the lower limit of normal (xLLN) with the 95% confidence intervals for the mean haemoglobin illustrated as shaded areas

The mean weight at baseline and at the end of the follow up period was higher for HIV-positive rather than HIV-negative patients in the matched sample, however the confidence intervals were very wide (see Figure 4-2). The mean weight gain over the course of treatment (from baseline to week 26) was 3.16kg for patients infected with HIV and 4.82kg among HIV-negative patients in the matched sample. HIV-positive patients had a mean weight of 55.81kg at the beginning of their treatment and 62.05kg at the end of follow-up (week 78), and this compared to a mean weight of 53.61kg at baseline and 61.19kg at week 78 in HIV-negative patients. The GEE regression coefficient of 1.78 was not significant (95% CI -1.87 – 5.43, p value 0.34).

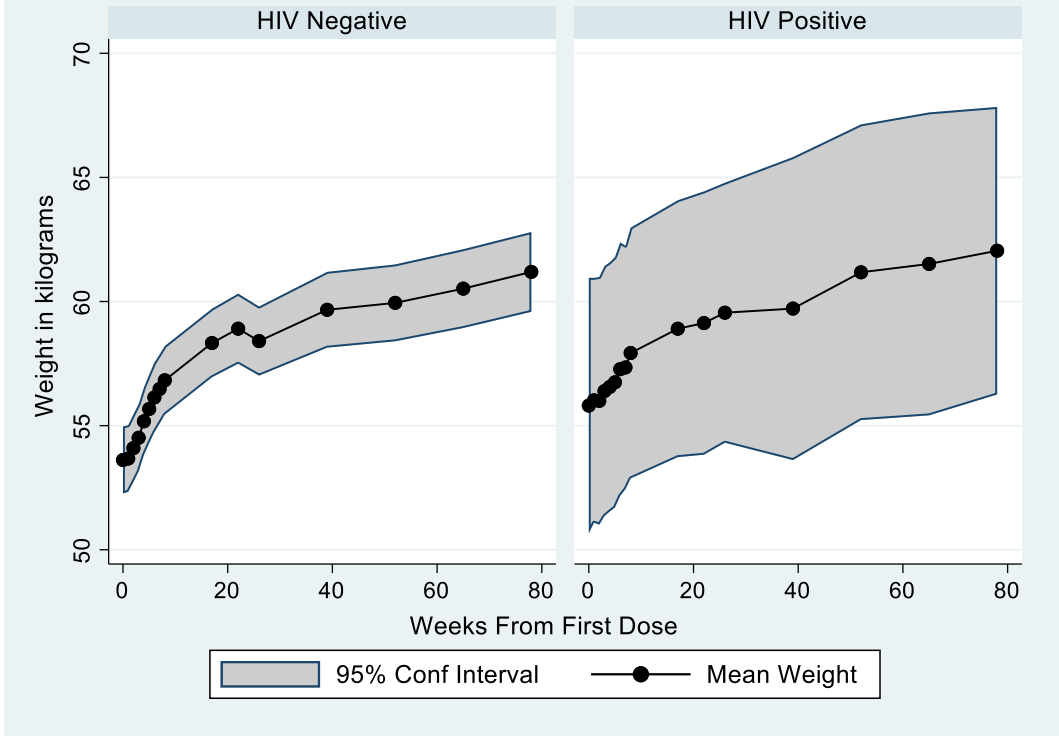

Figure 4-2. Longitudinal plots to present the mean recorded weight in HIV-positive and HIV-negative patients collected at protocol scheduled visits in REMoxTB. Weights obtained at unscheduled visits are not included in this graph, and weights are presented as kilograms with 95% confidence intervals for the mean value as shaded areas

Figure 4-3 shows that the mean number of TB symptoms reported reached a peak for both HIV-positive and HIV-negative patients between weeks 39 and 52 with approximately 10 symptoms reported on average per patient. There were 21 of 42 (50.0%) HIV-positive patients reporting ≥1 TB-related symptoms at baseline with a mean of 5.19 symptoms reported per patient. Among the HIV-negative patients, 126 of 220 (57.3%) reported ≥1 symptom in keeping with active TB and there was a mean of 4.60 symptoms recorded per patient. While the mean number of symptoms per patient rises to 6.00 at week 78 for the HIV-positive sample the total number of patients reporting symptoms drops over time and there were only 2 of 42 (4.8%) patients reporting TB symptoms at that visit. When adjusting for the interaction between weeks after treatment and HIV status, there was no significant association

between HIV-positivity and the number of TB symptoms reported in a GEE model with a coefficient of -1.76 (95% CI -1.10 – 0.75, p value 0.71).

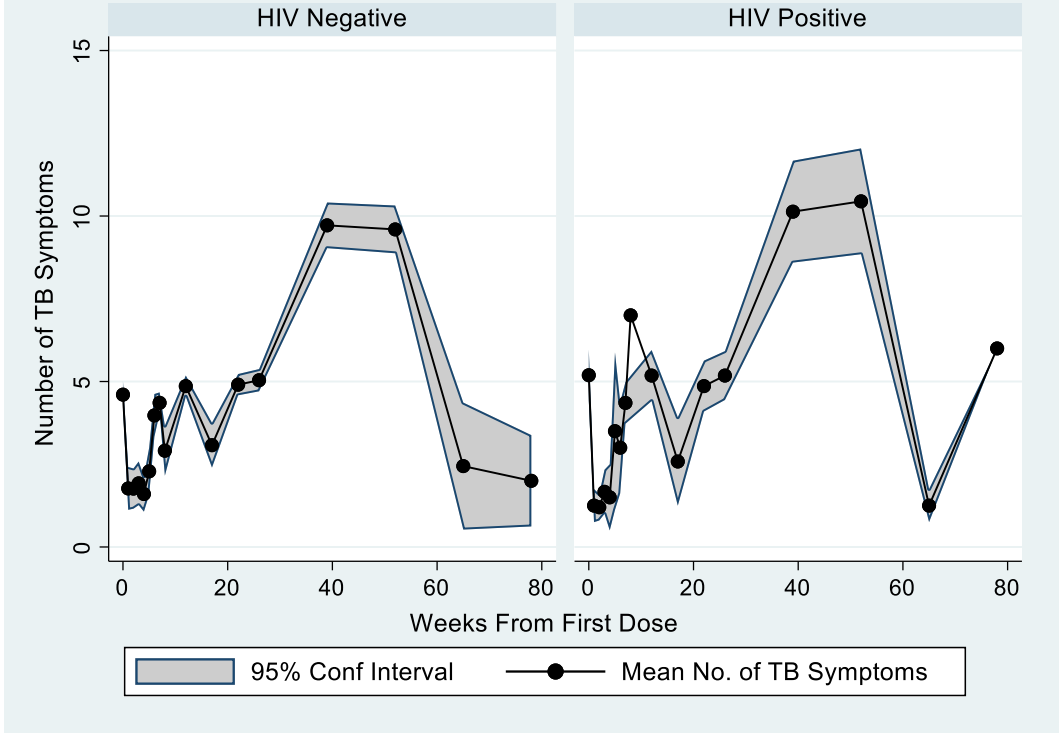

Figure 4-3. Longitudinal plots to present the mean number of TB-related symptoms reported by HIV-positive and HIV-negative patients collected at protocol scheduled visits in REMoxTB. Symptoms collected at unscheduled visits have been excluded from the data presented in the graph, and 95% confidence intervals are shown in grey either side of the line

During screening for the trial, chest pains and fever each accounted for 17 of 109 (15.6%) symptoms reported by the HIV-positive patients, with cough and breathlessness the second most frequent symptoms each accounting for 16 of 109 (14.7%) symptoms among those with HIV infection. Weight loss was 15 of 109 (13.8%) symptoms reported in the HIV-positive group. Cough (105 of 580, 18.1%), fever (81 of 580, 14.0%), chest pain (79 of 580, 13.6%), weight loss (70 of 580, 12.1%), and night sweats (65 of 580, 11.2%) were the five most common symptoms reported by the 220 HIV-negative patients.

Table 4-1 demonstrates the frequency of the reported symptoms among the 262 patients in the analysis along with percentages based on the total number of symptoms reported by these patients over the course of the trial.

TB Symptom	Frequency of Reported Symptoms	
	HIV-Positive (% of symptoms)	HIV-Negative (% of symptoms)
Cough	104 (18%)	555 (19%)
Chest pains	84 (15%)	420 (15%)
Shortness of Breath	76 (13%)	331 (12%)
Fever	73 (13%)	350 (12%)
Night Sweats	76 (13%)	351 (12%)
Weight Loss	63 (11%)	294 (10%)
Haemoptysis	36 (6%)	137 (5%)
Respiratory Abnormalities	13 (2%)	101 (4%)
Loss of Appetite	8 (1%)	82 (3%)
Fatigue	10 (2%)	62 (2%)
Other	21 (3.7%)	2692 (6%)

Table 4-1. The ten most frequently reported TB-related symptoms in the matched analysis sample of HIV-positive and HIV-negative patients. The number presented is the number of times a symptom was reported, and patients could not report a symptom more than once at a visit. Symptom count taken from all scheduled visits in the REMoxTB database for these patients, and unscheduled visits have been excluded. Percentages based on the total number of symptoms reported by these patients over the course of the trial. Percentage of all symptoms reported at scheduled visits accounted for by row, according to HIV status, is shown in brackets

There were 327 symptoms reported by the 42 HIV-positive patients over the course of their time in the trial; cough (25.7%), weight loss (13.8%), and chest pains (13.5%) were the three most common symptoms. There were 1,669 symptoms reported by the 220 HIV-negative patients in this analysis and cough (28.3%), chest pains (13.8%), and breathlessness (11.3%) were the most common. Weight loss only accounted for 131 of the 1,669 (7.9%) symptoms reported by the HIV-negative patients.

At their scheduled visits at week 8 and week 12, HIV-positive patients had a higher mean ALT result than those patients uninfected with HIV. The mean ALT result at week 8 was 1.10xULN and 0.68xULN for HIV-positive and HIV-negative patients, respectively. At week 12 the mean ALT was 1.25xULN for HIV-positive patients and

0.61xULN for HIV-negative patients and the mean ALT result at scheduled visits for both patient groups is presented in Figure 4-4. There was no statistically significant difference when the ALT results for the two patient groups were compared at week 8 and week 12 ($p > 0.40$ for both). The GEE regression coefficient of 0.03 for HIV status against ALT result was again not statistically significant (95% CI -0.21 – 0.27, p value 0.79).

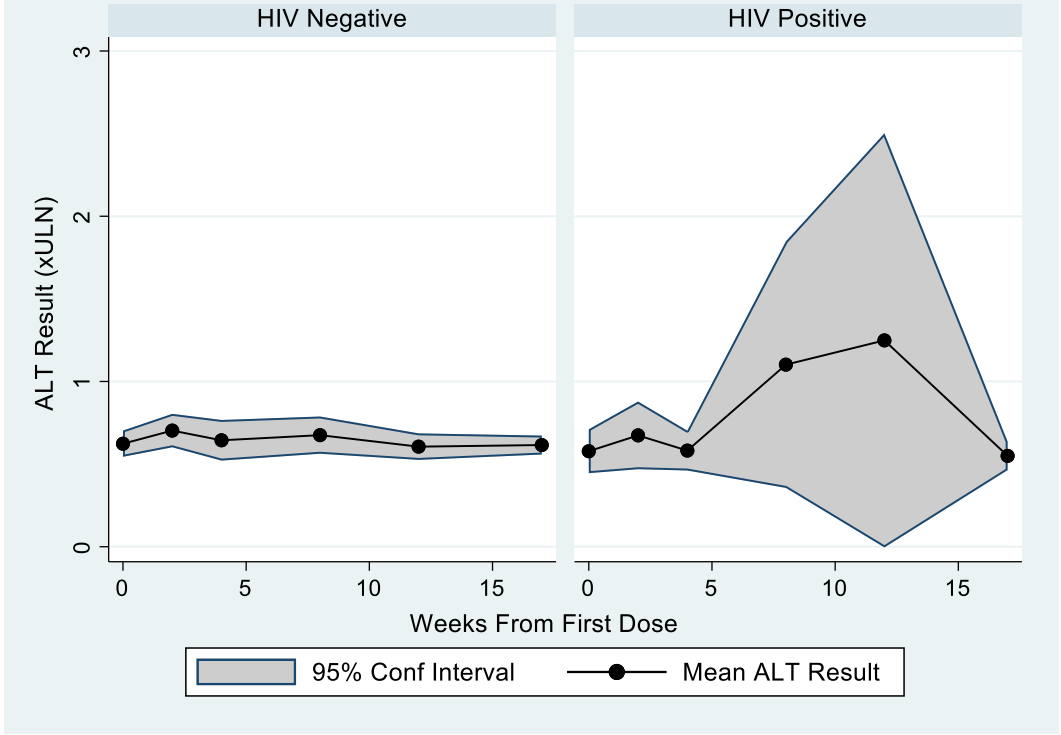

Figure 4-4. Longitudinal plot to present the mean value of the alanine transferase result in HIV-positive and HIV-negative patients taken at protocol scheduled visits in REMoxTB. Test results taken at unscheduled visits are not included in this graph, and ALT is presented as a multiple of the upper limit of normal (xULN) with 95% confidence intervals around the mean shown as shaded areas on the graph

Multivariate regression of the above variables at week 0 and week 17 against HIV status as an independent variable did not demonstrate any statistically significant associations (see Table 4-2). While it fell short of achieving the agreed level for statistical significance, the regression coefficient for the relationship between HIV status and both the week 0 and week 17 haemoglobin confirmed a negative

association as seen in the above longitudinal plot and the p values were very close to the 5% cut-off (0.07 & 0.06). All other variables were associated with a p value >0.10.

	Regression Coefficient	95% CIs	P value
Haemoglobin			
Week 0 Hb	-0.05	-0.10 - 0.00	0.07
Week 17 Hb	-0.05	-0.10 - 0.00	0.06
Weight			
Week 0 Wgt	2.57	-1.24 - 6.38	0.19
Week 17 Wgt	1.71	-1.98 - 5.40	0.36
Symptoms			
Week 0 Symp	0.04	-0.68 - 0.75	0.92
Week 17 Symp	-0.59	-1.37 - 0.18	0.13
ALT Result			
Week 0 ALT	-0.06	-0.22 - 0.10	0.45
Week 17 ALT	0.00	-0.22 - 0.21	0.97

Table 4-2. Regression coefficients from a multivariate model using Zellner's seemingly unrelated regression to test the effect of HIV infection on the dependent variables measured at Week 0 and Week 17. P values and 95% confidence intervals are provided for the model estimations

5 Treatment Outcomes in Matched Population Treated with Standard TB Therapy

Current guidance recommends an extension of treatment to 9 months in cases of TB-HIV co-infection when the patient is not receiving ART(Nahid *et al.*, 2016), however this is based predominantly on observational work and there has not been a systematic investigation of treatment outcomes in a prospectively recruited trial of these co-infected patients. A commitment to an extra three months of therapy in a group of patients with a greater chance of experiencing toxicity could present an unnecessary risk if they are as likely to achieve cure from 6 months of standard TB therapy.

This final line of inquiry drew on the available microbiological culture data from REMoxTB to elicit differences in treatment outcomes for HIV positive patients receiving standard TB therapy and a group of matched HIV negative controls. The aim

was to again take advantage of the systematic and detailed nature of the data collection in the trial to provide an evidence base for making decisions about TB treatment duration for ART-naïve TB-HIV co-infected patients.

5.1 Methods

The matched sample of HIV positive and negative patients receiving standard TB therapy was categorised according to their recorded outcome in the original REMoxTB publication. The definitions for favourable, unfavourable and unassessable outcomes in the original REMoxTB publication can be found in the Methods chapter. Per-protocol (PP) and modified intention-to-treat (mITT) population criteria are also described in detail in the Methods chapter. The proportion of HIV-positive and HIV-negative patients who met the criteria for different outcomes based on each of the analysis populations was determined and the Chi square test employed to assess the statistical significance of any differences in an isolated exploratory analysis.

The time to first sustained negative culture following randomisation was calculated for each patient in the matched sample. Sputum samples were collected at protocol-specified visits and at any unscheduled visits when it was thought necessary by the site doctor, and these samples were cultured in Mycobacterial Growth Indicator Tube (MGIT) as described in the Methods chapter. In the trial database, culture results have an associated date of collection and time-to-positivity (TTP). The number of weeks from the first dose of medication to sputum sample collection was calculated for each patient's culture results, and the "first sustained negative culture" was defined as the first culture with a subsequent negative culture collected a minimum of one week later with no positive cultures in between. The median time to sustained negative culture was calculated for HIV-positive and HIV-negative patients in the matched sample, and Kaplan-Meier curves were constructed for the time to first sustained negative culture

in both patient groups. Cox proportional hazards regression was used to obtain a hazard ratio for reaching a sustained negative sputum culture depending on HIV status in this matched sample.

Patients were then assigned into groups based on whether they achieved a microbiological cure. A composite culture status at eighteen months, requiring negative cultures on both Lowenstein-Jensen (LJ) slopes and MGIT culture to be considered culture negative, was available for the majority of patients randomised into the trial. In the same manner as the analysis in Chapters 3 and 4, patients who were culture negative at eighteen months were labelled as “cured”. If a patient was lost to follow up, or died before eighteen months in the trial, then they were considered cured if they had completed their treatment and had two or more consecutive negative cultures (at different visits) prior to the date that they were last seen. Those patients who were culture positive at eighteen months, or who were lost to follow up or died with less than two consecutive negative cultures immediately prior, were considered not cured.

5.2 Results

Among patients receiving standard TB therapy, 29 of 42 (69.05%) HIV-positive patients were favourable in both the PP and mITT assessments, along with 148 of 220 (67.27%) and 149 of 220 (67.73%) HIV-negative patients classed as favourable in the PP and mITT analysis, respectively. A higher proportion of matched HIV-positive patients were considered to have had an unfavourable outcome in both the per-protocol (PP) and modified intention-to-treat (mITT) populations for the original trial analysis in REMoxTB, however higher proportions of HIV-negative patients were considered un-assessable (Table 5-1).

Per-Protocol Population				
HIV Status	**Favourable**	**Unfavourable**	**Un-assessable**	**Total**
Positive (%)	29 (69.05)	3 (7.14)	10 (23.81)	42 (100.00)
Negative (%)	148 (67.27)	3 (1.36)	69 (31.36)	220 (100.00)
Modified Intention-to-Treat Population				
HIV Status	**Favourable**	**Unfavourable**	**Un-assessable**	**Total**
Positive (%)	29 (69.05)	4 (9.52)	9 (21.43)	42 (100.00)
Negative (%)	149 (67.73)	4 (1.82)	67 (30.45)	220 (100.00)

Table 5-1. Patient outcomes from the matched sample of HIV-positive and HIV-negative patients. The criteria for favourable, unfavourable, and unassessable are those used for the per-protocol and modified intention-to-treat analysis in the original REMoxTB publication. Row percentages are included to provide proportions of outcomes based on HIV status

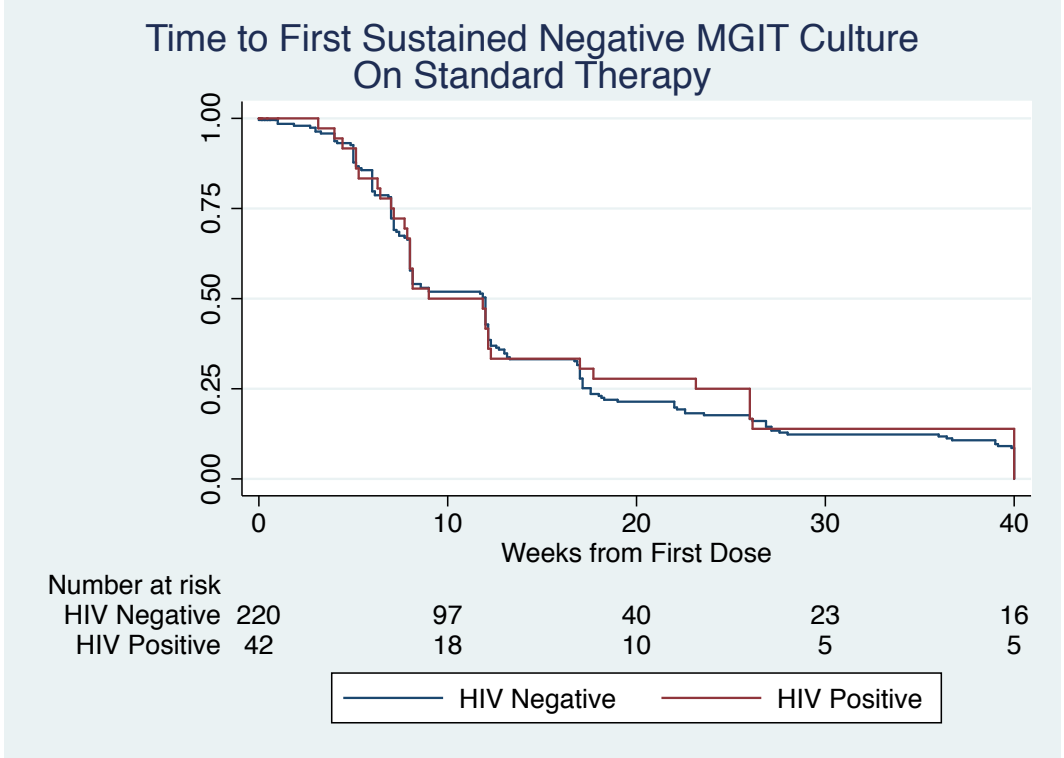

Figure 5-1. Kaplan-Meier curves illustrating the time to first sustained (two or more consecutive) negative MGIT culture for HIV-positive and HIV-negative patients in the matched sample.

The median time to the first sustained negative MGIT culture was 84 days (IQR 49 – 123) for HIV-positive patients and 73 days (IQR 49.5 – 172) in HIV-negative patients assigned to standard TB therapy. Figure 5-1 contains the Kaplan-Meier curves for time to first sustained (2 or more consecutive) negative MGIT culture. No significant difference was detected in the time to first sustained negative culture between HIV-positive and HIV-negative patients (logrank p value 0.60).

There were 4 of 42 (9.5%) of HIV-positive patients who failed to achieve microbiological cure as previously defined (see Section 4.1.1, Chapter 3), and 25 of 220 (11.4%) HIV-negative patients in the matched sample (p value 0.73). Table 5-2 contains the proportion of patients considered cured based on their HIV status. There were 4 of 38 (10.5%) HIV-positive and 8 of 195 (4.1%) HIV-negative patients who achieved microbiological cure referred to the National Treatment Program (NTP) at a median time of 41.5 days (IQR 19 – 53) and 130 days (IQR 93.5 – 150.5), respectively. “Adverse reaction/toxicity” was the reason given for withdrawal in 3 of the 4 (75.0%) HIV-positive patients and 3 of the 8 (37.5%) HIV-negative patients cured in the NTP. One HIV-positive patient was referred to the NTP after completing standard TB therapy in the trial for further treatment because of disease relapse; however, none of the HIV-negative patients were treated for relapse in this matched sample.

HIV Status	No Microbiological Cure	Microbiological Cure	Total
Positive (%)	4 (9.52)	38 (90.48)	42 (100.00)
Negative (%)	25 (11.36)	195 (88.64)	220 (100.00)

Table 5-2. *Table presenting the proportion of HIV-positive and HIV-negative patients recorded as achieving microbiological cure at 18 months based on culture data from the REMoxTB database. Row percentages provided to give proportion of HIV-positive and HIV-negative patients classified as cured, and Chi square p value 0.73*

6 Discussion

This chapter of the thesis investigated the incidence of toxicity in HIV-positive patients with high CD4+ counts and not receiving ART, and matched HIV-negative controls. All the results presented must be interpreted in light of the fact that they relate to these, presumably recently diagnosed, HIV-positive patients and are not generalisable to HIV-positive patients as a whole. These findings demonstrated that in a matched sample of ART-naïve HIV-positive patients with higher CD4+ counts there were still

fewer cavities on chest X-ray at presentation. Although there was no statistically significant difference detected in the variation of selected clinical parameters during treatment, the HIV-positive patients were more likely to begin and end treatment with a haemoglobin result below the lower limit of normal. These HIV-positive patients receiving standard TB therapy in the REMoxTB trial were at greater risk of toxicity during treatment but not at greater risk of failing to achieve microbiological cure, when compared to the matched sample of HIV-negative patients receiving the same treatment.

Cavitation on the chest X-rays of patients diagnosed with pulmonary TB has been shown to act as a marker of bacillary burden during the infection (Ong, Elkington and Friedland, 2014). HIV-positive patients more frequently present with pauci-bacillary disease and extra-pulmonary TB (Swaminathan, Padmapriyadarsini and Narendran, 2010; Maartens, Celum and Lewin, 2014; Bell and Noursadeghi, 2017), and as such observational studies have demonstrated higher rates of patients presenting with either fewer cavities or no cavitation when compared to HIV-negative TB patients (Pitchenik H A, 1985; Pedro-Botet *et al.*, 1992; Post, Wood and Pillay, 1995). The downregulation of CD4+ cells and macrophages seen in HIV infection impairs the host's capability to contain the infection, and a consequent disruption of granuloma formation in the lung tissue followed by expansion of the mycobacterial population (Shankar *et al.*, 2014; Hunter, 2016; Bell and Noursadeghi, 2017). This analysis detected a significant trend for active TB without cavitation at presentation for HIV-positive patients when all patients were considered, but this trend was no longer significant when only the matched population was considered.

Further to this point, it is noteworthy that the HIV-positive patients in both the total and matched samples did not differ significantly from the HIV-negative patients in the time

to positive result (TTP) of MGIT sputum culture at baseline. The similar TTP for these ART-naïve HIV-positive with higher CD4+ counts and HIV-negative patients presented here, along with their response to treatment, would suggest comparable burden of disease in both groups and argue against the accepted maxim that all HIV-positive patients present with less of a bacillary load. While there should be a degree of caution before applying these results to the wider HIV-positive population, as there is a spectrum of disease severity predominantly according to the CD4+ count (Ahmad Khan *et al.*, 2012; Ismail and Bulgiba, 2013; Lawn *et al.*, 2013; Pepper *et al.*, 2015), some conclusions can be drawn relating to the presentation of HIV-positive patients with HIV disease that is not easily clinically detectable. These findings should dispel any complacency that a patient presenting with classical pulmonary TB is less likely to have HIV co-infection, and confirms the importance of testing all TB patients for HIV infection. This is most relevant in resource-limited settings, where constrained access to HIV testing can lead to a rationing for cases with the highest clinical suspicion, and there is a need for more evidence that will inspire direction of more funding and resources globally.

Chest pains, fever, cough, and breathlessness were the four most common symptoms reported in the HIV-positive patients at screening. These four symptoms collectively made up 61% of the symptoms reported, and weight loss accounted for only 14% of the screening symptoms reported among the HIV-positive group. The WHO recommends routine screening of HIV-positive patients for active TB using a clinical algorithm of one or more of the following symptoms: cough, fever, weight loss, or night sweats (WHO 2012; WHO 2011). In a meta-analysis reviewing the individual patient-level data of HIV-positive patients with pulmonary TB, the best-performing diagnostic strategy was the presence of any one of these four symptoms (Getahun *et al.*, 2011).

This meta-analysis demonstrated a sensitivity of 79% and a specificity of 50% after including data for over 8,000 patients, and the negative predictive value was 90% at 20% prevalence of TB among HIV-infected individuals. An issue that arises from such low sensitivity and specificity is that of missed diagnoses of active TB (as well as incorrect diagnosis of TB) among HIV-positive patients in areas with a higher prevalence of TB, such as South Africa. In this chapter, among the HIV-positive patients only cough and fevers (both daytime and night time) were reported from the four most common symptoms, and weight loss was a small fraction of the symptoms at presentation recorded in the database. If the WHO criteria were stringently applied to these patients in a setting with no access to other investigations, then there could be a real danger of missing the diagnosis of active TB and delaying treatment as a result.

The GeneXpert platform allows for the rapid identification of *M tuberculosis* in sputum (and other bodily fluids) with a sensitivity of up to 75% even in cases of sputum smear-negative pulmonary TB (Boehme *et al.*, 2010; Steingart *et al.*, 2014), and this also provides molecular results for the rifampicin resistance profile of the organism. The WHO and other policy-makers now recommend GeneXpert as a first-line method for diagnosing active TB (WHO, 2013; Lewinsohn *et al.*, 2017); however the infra-structure required to support this technology has acted as a barrier to widespread implementation. The symptom data collected as part of REMoxTB acts as yet more evidence that infra-structure development to allow the use of existing, validated diagnostic technology in poverty-stricken areas must be a priority (Albert *et al.*, 2016). Rapid identification and treatment of pulmonary TB leads to reduced time for the patient to act as an infectious carrier of the disease and earlier initiation of treatment

is associated with improved outcomes (Ormerod and Prescott, 1991; Chee *et al.*, 2000).

HIV-positive patients in this sample were treated with standard TB therapy lasting six months, and there was no significant difference in the proportion of patients who were cured 18 months after randomisation. The World Health Organisation (WHO) TB treatment guidelines recommend that TB-HIV co-infected patients should receive at least the same duration of TB therapy as those patients who are not infected with HIV (WHO 2011). The WHO note the results of the 2010 systematic review by Khan et al that demonstrated lower rates of recurrence in HIV-positive patients treated for 8 or more months (Khan *et al.*, 2010), however their assessment was that the data quality for the included studies was low and that extending treatment based on HIV status could be operationally challenging. The most recent guidance from the Infectious Diseases Society of America (IDSA) recommends extending TB treatment to 9 months in cases where HIV-positive patients are not taking ART to reduce the risk of treatment failure or relapse, and to treat with standard TB therapy for six months if a patient is receiving ART (Nahid *et al.*, 2016).

The findings in this chapter support the use of 6 months' standard TB therapy in HIV-positive patients, as recommended by the WHO (WHO 2011), in cases with higher CD4+ counts if the patient is not taking ART. The predominant concern in treating ART-naïve TB-HIV co-infected patients for 6 months is the risk of treatment failure or relapse (Nettles *et al.*, 2004; Weiner *et al.*, 2005; Nahid *et al.*, 2016), however in this analysis there was only one case of disease relapse meriting re-treatment following standard TB therapy. Additionally, the HIV-positive patients who achieved a microbiological cure were referred to the NTP prior to completing treatment in the trial at a median time of 41.5 days and the most common reason was toxicity or a

complication during treatment. Therefore, the patients in the trial who required additional treatment were identified at an early stage. The WHO guidance raises the point of logistical challenges surrounding an extension of treatment in HIV-positive individuals (WHO 2011), and this data would indicate that a more appropriate method might be to ensure that HIV-positive patients are monitored closely during the intensive phase (as mentioned in previous chapters, and given that toxicity was the most common reason for withdrawal). Those patients who complete the intensive phase of treatment uneventfully could then perhaps be treated with standard TB therapy for six months, and this would reduce unnecessary burden on TB treatment programs that are already under-resourced.

Coarsened exact matching (CEM) was adopted to address the limitations of the small numbers in this analysis by trying to create a case-control sub-study from the trial data. The analysis of small samples is fraught with danger that effect size will either be underestimated or overestimated due to undetected sampling bias as small numbers will frequently over-represent one aspect of the sample population (Lagakos, 2006; Chisti *et al.*, 2015; Shann and Lange, 2015). The principle of regression to the mean demonstrates the effect on sample characteristics as the size of the sample increases and the sample mean will be brought to a place more representative of the true population mean. Coarsened exact matching seeks to improve the balance in the data between treatment and control groups (in this case the HIV infected and uninfected), leading to more similar empirical distributions in the two groups of the covariates used in the matching process. By breaking the covariates of interest down into bins, CEM approximately matches each HIV-positive patient with at least one HIV-negative control and this leads to less model dependence and reduced statistical bias when the two groups are compared. This does not completely remove the caveats that must be

applied when dealing with sample sizes as small as the HIV-positive sample in this analysis (Lagakos, 2006; Wang *et al.*, 2007; Button *et al.*, 2013), however it does allow for some meaningful conclusions to be drawn. The use of CEM to create the case-control design should help to reduce the bias that will be inherent in this post-hoc (essentially observational) analysis of the trial data and re-inforce the significance of the differences noted between the two groups within the confines stated throughout the chapter.

6.1 Limitations

A further limitation to this analysis is the exclusion of HIV-positive patients with a CD4+ count less than 250 cells/mm^3 by the trial protocol. These ART-naïve patients with HIV infection and high CD4+ counts represent a group that have less advanced disease, and this means that the rates of adverse events should be considered to represent a favourable scenario for patients not receiving ART. This should also be noted when considering the data on clinical change over time and microbiological outcomes. Unfortunately, this means that broader conclusions about ART-naïve TB-HIV patients cannot be drawn from the analysis presented here as these patients will frequently present to healthcare at a later stage with HIV disease that is more advanced and lower CD4+ counts. ART has been conclusively shown to improve TB-HIV co-infected patients' outcomes, and this chapter does not disagree with the need for early ART in these patients despite the reassuring treatment outcomes.

6.2 Conclusions

This chapter concludes the investigation into toxicity during treatment for pulmonary TB by performing a detailed examination of the nature of adverse events experienced by HIV-positive patients in the REMoxTB trial, a patient group shown to be at

significantly greater risk of toxicity in the two previous chapters. The increased risk of adverse events associated with HIV infection has implications for clinical monitoring of these patients and confirms the need for clinicians to ensure that TB programs focus on this high-risk group even in cases where the CD4+ is preserved. The treatment outcomes among the HIV-positive and HIV-negative patients were similar with six months of standard TB therapy, and those patients who required longer durations of therapy were identified early in treatment. TB therapy is particularly toxic for HIV-positive patients, and this would indicate that those patients performing well clinically do not need to be exposed to a longer duration of these hazardous drugs. The data presented in this chapter illustrates the wealth of information that can be gained from even a small number of patients prospectively recruited and systematically followed over the course of treatment. However the management of HIV has significantly changed over the past five years and the goal is now to ensure that all HIV-positive patients are initiated on ART much earlier, therefore limiting the generalisability of these findings. In future Phase III TB trials it will be critical that recruitment and monitoring of HIV-patients is undertaken with sufficient accuracy to translate into real clinical benefit for this often under-served patient population.

Chapter Six: Discussion

1 Toxicity Related to Tuberculosis Treatment

The toxicity associated with any treatment works to undermine its effectiveness, and this holds true with treatment-related toxicity in tuberculosis (TB) patients. There is a cure available for this disease, and yet it remains one of the leading causes of death globally (WHO, 2017). This state of affairs is clearly not wholly attributable to the treatment interruptions and associated increased morbidity and mortality seen with TB treatment-related toxicity (Saukkonen *et al.*, 2006; Sharma *et al.*, 2010; Shang *et al.*, 2011); however, toxicity is likely to be a contributing factor.

The work presented in this thesis was intended to characterise the nature of toxicity related to standard treatment for drug-sensitive pulmonary TB, and the observed impact on treatment outcomes, based on data from the REMoxTB study. Additionally, the toxicity associated with the experimental, moxifloxacin-containing treatment arms was investigated and compared to that seen on standard TB therapy. The intentions were to better inform clinicians as to the true side effect profile of standard TB therapy, contribute to the development of monitoring programs, and help inform the current research agenda through a better understanding of the shortcomings of existing TB treatment.

1.1 Who is at Risk of Toxicity on Standard TB Therapy?

Female patients, those infected with the human immunodeficiency virus (HIV), and patients of Asian ethnicity were found to be at significantly higher risk of experiencing toxicity. Approximately 10% of patients taking standard TB therapy experienced one or more clinically significant adverse events that were thought to be related to

treatment, with female and HIV-positive patients at significantly greater risk in Chapter Three. The analysis in Chapter Four demonstrated that around 6% of all patients experienced a clinically significant elevation of their liver enzymes while receiving standard TB therapy, and patients classed as Asian ethnicity were at significantly higher risk of hepatotoxicity. Asian ethnicity has been previously linked to increased risk of hepatotoxicity (Huang, 2014; P. Wang *et al.*, 2016), thought to be contributed to by the high proportion of patients of slow-acetylator status among some populations in Asia (An *et al.*, 2012; Chan *et al.*, 2017), and these results reinforce the need to ensure that these patients are monitored closely during treatment with standard TB therapy. However, a significant association between female sex and toxicity related to standard TB therapy has only been mentioned in a small number of publications (Ormerod and Horsfield, 1996; Yee *et al.*, 2003; Marra *et al.*, 2007), and this could be related to the higher reported incidence of pulmonary TB among males (WHO, 2017) leading to smaller numbers of females with fewer events observed. While the findings presented here indicate a novel finding of higher risk of adverse events among females, it also needs to be remembered that adverse events are subject to reporting bias (McGauran *et al.*, 2010). Therefore, it is not clear whether it is a matter of genuinely increased incidence of toxicity, or increased identification and reporting of adverse events.

Interestingly, a lower baseline weight, indicators of higher bacillary load (time to positive Mycobacterial Growth Indicator Tube result (TTP) and cavitation on chest X-ray) and current or previous smoking status were not significantly associated with an increased risk of adverse events or toxicity in a logistic regression analysis in Chapter Three. This is a finding that stands in contrast to previously published work where associations have been made between increased rates of treatment-related

complications and poor baseline health or higher bacillary load (Chang, Leung and Tam, 2004; Leung, Chan and Yew, 2004; Forget and Menzies, 2006; Mouton *et al.*, 2018). The enrolment of patients into the trial using strict inclusion and exclusion criteria lends strength to the results of analyses using the REMoxTB database by helping to define the patient population clearly, and therefore focussing the conclusions on these patient groups who were also closely monitored as part of the trial. These baseline findings could be related to other factors such as delay in seeking medical attention and generally negative aspects of the patient's lifestyle; these could be the actual drivers behind the association seen in observational studies. The inclusion and exclusion criteria of the trial acted to select out patients who were more unwell at baseline and therefore the association seen in observational studies could have been lost.

The HIV-positive patients in the study were enrolled with higher CD4+ counts and all, except for 2 patients, were not taking anti-retroviral therapy during TB treatment. Previous observational work has attributed varying levels of risk for adverse events during standard TB therapy for HIV-infected patients (Yee *et al.*, 2003; Dworkin *et al.*, 2005; Lorent *et al.*, 2011; Tesfahuneygn, Medhin and Legesse, 2015), and this work provides insight into a sub-group of these patients. In particular, there has been an association noted with hepatotoxicity on treatment which is sometimes reduced or increased in the context of ART (Saukkonen *et al.*, 2006; Yimer *et al.*, 2008; Pasipanodya and Gumbo, 2010; Lorent *et al.*, 2011). While HIV-positive patients were at significantly higher risk of toxicity in general in this analysis (found in Chapter Three), the risk was attenuated when only clinically significant liver enzyme elevations were considered (15% vs 9% for HIV-positive and HIV-negative patients in Chapter Four, respectively). This is likely to have been influenced by the small numbers,

relative good health of the patients, and the lack of overlapping toxicity from anti-retroviral therapy (ART). Hence, it is still noteworthy that the proportion of patients affected by hepatotoxicity is almost double in the HIV-positive group.

1.2 What is the Nature and Timing of the Toxicity Associated with Standard TB Therapy?

Hepatotoxicity was the most common form of treatment-related toxicity detected among patients receiving standard TB therapy, as seen in Chapter Three and explored further in Chapter Four. Toxicity affecting the liver has been identified as a common occurrence with standard TB therapy in earlier reports and the safety findings of the original clinical trials when short-course chemotherapy was developed (Fox, Ellard and Mitchison, 1999). The reported incidence of toxicity in the existing literature is wide, and ranges from approximately 5-30% (Thompson *et al.*, 1995; Tost *et al.*, 2005; Saukkonen *et al.*, 2006; Shu *et al.*, 2013) of treated cases but the incidence in the REMoxTB study was closer to the lower limit of this range. While approximately 6% of patients experienced clinically significant liver enzyme elevations (a three-fold or greater rise above the upper limit of normal), it was noteworthy that only 3% of patients met the criteria for true drug-induced liver injury (DILI). Several reasons could explain this lower incidence, compared to the apparently higher rates of toxicity seen in other work. First, each of these patients was prospectively followed at frequent reviews at protocol-scheduled assessments and therefore treatment may have been interrupted earlier than would normally happen in normal practice. Second, the definition of "hepatotoxicity" varies between these reports and could be considered an asymptomatic liver enzyme elevation, enzyme elevations at varying cut-off values, or only patients who develop symptoms that prompt liver biochemical testing. Third, inclusion and exclusion criteria were applied to select out a less unwell patient

population and therefore this incidence should be considered a minimum. Finally, this study involved patients recruited at different sites across the world and is more of a generalised estimate. Many of the published reports involve only one ethnic group and this may at least partly explain the discrepancy seen, bearing in mind the significant association seen between Asian ethnicity and hepatotoxicity in the REMoxTB study.

The majority of all adverse events occurred in the first two months of therapy, and over 50% of the total related adverse events reported by patients receiving standard TB therapy were accounted for by hepatobiliary disorders and musculoskeletal disorders (predominantly arthralgia). The role of pyrazinamide in standard TB therapy during the first two months of therapy is essential to ensure adequate treatment in six months (British Medical Research Council, 1974; Grosset, 1978; Association, 1982; Snider *et al.*, 1984), but there is no doubt that it is also a toxic drug (Horsfall *et al.*, 1979; Jenner *et al.*, 1981; Steele and Des Prez, 1988; Schaberg, Rebhan and Lode, 1996; Jasmer *et al.*, 2002; Lee *et al.*, 2002; Yee *et al.*, 2003). The earliest studies demonstrated the hepatotoxic potential of pyrazinamide and this has also been found to be both dose-related and idiosyncratic (British Medical Research Council, 1981; Hong Kong Chest Service/Tuberculosis Research Centre, 1989; Jindani, Nunn and Enarson, 2004; Chang *et al.*, 2007). The pattern of toxicity in this analysis would seem to reinforce pyrazinamide's standing as one of the drugs in the regimen with the most pronounced side effect profile, and acts as a reminder that standard TB therapy continues to rely on drugs that are poorly tolerated and only remain in use because of a lack of viable alternatives.

1.3 What is the Impact of Toxicity on Treatment Outcomes?

Patients who experienced one or more adverse event had approximately 3-fold greater odds of failing to achieve a microbiological cure on standard TB therapy, for

both total adverse events and those assessed as being related to treatment only. Toxicity related to treatment is associated with higher rates of treatment failure in tuberculosis (Ormerod and Prescott, 1991; Pablos-Méndez *et al.*, 1997; Chee *et al.*, 2000) and other infections as well (Shields *et al.*, 2016; Danel *et al.*, 2017), and this relationship is generally attributed to interruptions in treatment that occur because of the toxicity. However, the relationship is likely to be more complicated than this; while the odds ratio was higher when only related events were included, the odds were still significantly elevated when all events were included. Therefore, this data would seem to indicate that sometimes a treatment failure may be heralded by non-specific symptoms and findings that actually represent the failure itself in a less-defined form, and also that adverse events on treatment are markers of increased risk of treatment failure or recurrence through several potential mechanisms (including toxicity).

The social aspect of TB disease is well-recognised: poverty and marginalisation are characteristic of the typical TB patient population (Lö *et al.*, 2009; Lönnroth *et al.*, 2010; Wingfield *et al.*, 2018) and a failure to deal with the broader issues underlying the disease presentation can mean that even successful microbiological cures ultimately end in a poor outcome. Poverty is associated with increased risk of other illness, both infectious and non-communicable (Pickett and Wilkinson, 2015; Chetty *et al.*, 2016; Canudas-Romo, 2018; Chokshi, 2018), and violence (Krug *et al.*, 2002; Sen, 2008) that contribute towards a lower life expectancy among affected individuals. The majority of patients who died on the standard TB therapy arm in the study did so having completed their treatment, for reasons both related and unrelated to their pulmonary TB. The finding of more deaths occurring after treatment had been successfully completed (often with no clinically significant adverse events reported), and within 18 months of their randomisation, should give some pause for thought. There is evidence

available demonstrating that simple but effective interventions that impact on both the risk of TB disease (Lönnroth *et al.*, 2010; Wingfield *et al.*, 2018) and violence (WHO 2014) are possible, but what is required is the political will to bring about change. While the need for novel shorter, more effective treatment for TB is apparent the REMoxTB mortality data is a reminder that a holistic view of the patient and their circumstances is necessary to achieve a truly successful outcome following treatment.

1.4 What is the Toxicity Profile of the Experimental Moxifloxacin-containing Treatment Arms?

The intention behind investigating the experimental arms was to better understand what place they could potentially have in TB treatment at a time when new treatment options are desperately needed. The work presented in this thesis has shown that the experimental treatment arms were less toxic than standard TB therapy among the trial population in the REMoxTB study, although this must be considered in light of the fact that these regimens failed to demonstrate non-inferiority compared to standard TB therapy in terms of treatment outcomes. A question that hangs over these experimental regimens relates to what the optimum duration of treatment is for them; a less toxic treatment option that can be delivered in six months with outcomes comparable to standard TB therapy could still have an application in treating high-risk patients, for example.

Isoniazid is used in isolation to treat latent TB infection, and because of this it is recognised as being a hepatotoxic drug. The rate of liver dysfunction while taking isoniazid preventive therapy is reported as ranging between 0.1 – 5% (Nolan, Goldberg and Buskin, 1999; Jasmer *et al.*, 2002; Al-Darraji, Kamarulzaman and Altice, 2012)and both the British and American Thoracic Societies (Ormerod *et al.*, 1998; Saukkonen *et al.*, 2006) recognise the hepatotoxic potential of the drug when advising

on the need for treatment interruptions. As mentioned in Chapter Four (Section 8), after unacceptably high rates of hepatotoxicity were observed from the combination of rifampicin and pyrazinamide used to treat latent TB infection (Jasmer *et al.*, 2002; Stout *et al.*, 2003) the theory that isoniazid may exert some kind of protective effect was raised (Lee *et al.*, 2002; Hest *et al.*, 2004). The work presented in Chapter Four would disagree with this proposal and support the hepatotoxic nature of isoniazid. The isoniazid-containing regimens demonstrated higher rates of drug-induced liver injury (DILI, 3.4% on both standard TB therapy and the isoniazid arm, compared to 2.2% on the ethambutol arm). Furthermore, patients receiving an isoniazid-containing regimen also reached a clinically significant liver enzyme elevation faster than those patients on the ethambutol arm (18.5 vs 28 days). In cases of significant hepatotoxicity on standard TB therapy isoniazid should remain a potential culprit drug and its exclusion from liver-sparing regimens is justified by these analyses. Lastly, this also highlights the potential role of the ethambutol arm in the treatment of TB patients at high risk of hepatotoxicity, if a duration that makes the regimen more efficacious can be defined.

Moxifloxacin has previously been shown to have a favourable safety profile in earlier studies that predominantly relate to its use in bacterial infections (Gillespie, 2016). However, it is still infrequently associated with cardiac arrhythmias (Haverkamp *et al.*, 2012; Mehrzad and Barza, 2015), dysglycaemia (WHO 2014), tendinopathies (van der Linden *et al.*, 2002; Khaliq and Zhanel, 2003), and very rarely hypersensitivity-mediated fulminant liver failure (Soto *et al.*, 2002; Nori *et al.*, 2004; Verma *et al.*, 2009). In light of these accepted toxic effects, there was some uncertainty around the potential for an increased duration of treatment leading to an increased risk of toxicity when moxifloxacin was proposed as a treatment for TB. However, the number of patients experiencing one or more events was lower in both the isoniazid (36 patients)

and ethambutol (25 patients) arms compared to standard therapy (47 patients). The original REMoxTB publication noted that there was no increased incidence of fluoroquinolone-associated toxicity on the experimental arms (Gillespie *et al.*, 2014) (e.g. tendinopathy, dysglycaemia, cardiac arrhythmias), and a further examination of the safety data relating to the trial corroborated this finding. There were also fewer patients with clinically significant liver enzyme elevations (ALT and/or AST ≥3xULN) among those receiving the experimental, moxifloxacin-containing regimens compared to standard TB therapy (7.0% vs 9.4%) although this must be interpreted in light of the similar incidence of DILI on standard TB therapy and the isoniazid arm. Overall, the REMoxTB database supports the role of moxifloxacin in the treatment of pulmonary TB, and this is an encouraging finding given the drug's role in the treatment of both isoniazid-monoresistant and rifampicin-resistant TB.

2 Applying the Results to Practice

2.1 Patients with Pulmonary Tuberculosis

A clear understanding of the expected toxicity associated with standard TB therapy will help with informed decision-making, and it is hoped it will improve adherence to treatment. Patients who are aware of the true frequency of adverse effects from the medication can be prepared for these toxicities, and if they do occur there will be less surprise in the context of an informed agreement to treatment. Previous work has shown that this has a positive impact on adherence to treatment and consequently a positive effect on treatment outcomes as well. Empowerment of patients with more knowledge about the treatment they are to receive, with a rational discussion around the risk to benefit ratio, is one of the goals of modern medicine and this work will contribute to this ongoing effort.

Patients taking standard TB therapy must be aware of when it is necessary to seek medical attention. As well as informing patients of the less serious toxicity associated with treatment to attempt to improve adherence, they must also be aware that some toxicities are a cause for concern and require intervention. The analyses presented here provide reassurance that many side effects from standard TB therapy are not life-threatening; however, there were several cases of extremely high liver enzyme elevations with associated clinically symptoms of gastrointestinal upset and abdominal pain. Drug-induced hepatotoxicity can be a fatal complication and, while there were no confirmed deaths due to drug-induced hepatotoxicity in this study, it is critical that patients are aware of the symptoms of hepatotoxicity and the importance of prompt medical intervention.

2.2 Clinicians Managing Pulmonary Tuberculosis

The information in this thesis adds to the evidence base to assist clinicians in making informed decisions regarding the monitoring of at-risk individuals, the interpretation of findings and the management of toxicity. The identification of increased risk of toxicity among female patients, those with HIV infection, and Asian patients allows TB clinicians to easily target patients who are at greater risk of a complicated treatment course and take appropriate steps (tailored to the individual patient's situation) to ensure an adequate package of care is in place. A proposed adequate care package would involve the direction of resources to ensure that any barriers to a patient attending for monitoring visits are addressed (for example housing or transport costs) as well as ensuring the availability of the materials and equipment (such as laboratory facilities). While not every patient with these baseline characteristics will experience clinically significant toxicity, it is hoped that an increased awareness of these patient

groups would result in toxicity being addressed in a timely fashion and liver toxicity is one of the clearest examples.

The interpretation and appropriate response to liver function tests during treatment with standard TB therapy is a point of contention. The expected pattern of liver enzyme elevations in all patients receiving standard TB therapy, including those without any symptoms of hepatotoxicity, is not well defined and the official guidance from the American and British Thoracic Societies provides advice on treatment changes using liver enzyme levels that have been arbitrarily assigned (3x and 5x the upper limit of normal, with and without symptoms or elevated bilirubin). The data presented here demonstrated that the majority of patients with liver enzyme elevations of 3xULN or higher completed standard TB therapy and were not withdrawn from the trial. Additionally, only a small proportion of patients developed liver enzyme elevations greater than 5xULN and the practice was to re-introduce all drugs in the regimen at the same time in the trial.

This would suggest that a practice of close monitoring but continuing treatment when liver enzyme levels are >3xULN but <5xULN would be reasonable in absence of concerning features, and adopting a re-introduction of the full regimen would also be reasonable. Furthermore, the small proportion of patients with liver enzyme elevations >5xULN should prompt the TB physician to think broadly and include other potential causes of hepatotoxicity in their differential diagnosis instead of automatically concluding that standard TB therapy is the underlying cause.

2.3 Policy Makers Directing Tuberculosis Services

Episodes of clinically significant toxicity were associated with a statistically significant increase in the odds of a patient failing to achieve a cure in the REMoxTB study. The

public health and economic implications to these findings should be given appropriate consideration by policy makers. Decisions regarding the allocation of resources are weighted by the demonstrated benefit or consequences of changing practice or failing to address certain issues; these results make the benefit of adequately supporting TB services more apparent.

Patients who fail to be cured of their TB can either remain infectious or return to an infectious state, are at a higher risk of developing drug-resistant infections, and also are unlikely to remain fit to continue working. The first two risks are of public health concern as they will directly work against any efforts to curb or eradicate TB in a region, and the third a well-recognised economic issue associated with TB infection. Policy makers working in TB endemic areas are often faced with difficult decisions about resource management, and this helps to provide an evidence-based rationale to give attention to supporting TB services to not only supply treatment but also in the monitoring and management of complications. This thesis has indicated that the majority of liver enzyme elevations and cases of drug-induced liver injury occur in the intensive phase of treatment. The incidence of significant liver enzyme elevations in the intensive phase may help to guide the timing for patient monitoring and provides some reassurance to the clinician that patients who successfully complete the intensive phase of treatment are less likely to develop significant hepatotoxicity. However, the optimum monitoring schedule for drug-induced liver injury remains unclear.

2.4 The Potential Role of the Moxifloxacin-containing Regimens

The experimental treatment arms had fewer patients reporting clinically significant adverse events compared to the standard TB therapy arm. The main implication of

this finding would be the potential for the moxifloxacin-containing regimens to be adopted as treatment options for patients who are at elevated risk of experiencing adverse effects from treatment (for example, those with advanced liver disease, other significant pathologies, or an intolerance of isoniazid). However, the duration would clearly have to be extended given that four months of treatment was not non-inferior to standard TB therapy, and it also cannot just be assumed that six months of therapy with the experimental regimens would be sufficient. The inclusion and exclusion criteria for REMoxTB resulted in the recruitment of relatively healthy patients, and therefore the toxicity seen with standard TB therapy in these analyses should be seen as a minimum incidence. This may have also acted to close the gap in the incidence of toxicity on standard TB therapy and the experimental treatment arms.

As a result, questions arise as to whether these treatment arms could potentially have a place in the treatment of TB for patients at greater risk of treatment-associated toxicity (e.g advanced liver disease, other significant chronic diseases, or intolerance to isoniazid or ethambutol). However, any possible benefits of using these less toxic regimens must be carefully balanced against their failure to achieve formal non-inferiority compared to standard TB therapy, the uncertain optimal duration of these experimental arms, and that with close monitoring in a clinical trial setting less than 10% of patients taking standard TB therapy were affected by significant toxicity. Therefore it should be considered that, rather than introducing the experimental arms as a novel treatment regimen for drug-susceptible TB, the best course of action could be simply working to increase the resourcing of existing TB services to allow them to deliver standard TB therapy in a setting that helps with early detection of adverse events.

3 Future Directions

3.1 Development of Novel Tuberculosis Treatments

There is a clear need for less toxic treatments for tuberculosis. In this thesis, the incidence of clinically significant toxicity was between 10-20% on standard TB therapy and there was a significant impact on treatment outcomes. In the early development of new TB treatment candidates there is much emphasis placed on the efficacy of a new regimen, and rightly so. However we must be careful to not be lulled into a false sense of security that a novel regimen as toxic as standard TB therapy has an acceptable safety profile. The comparator for these new regimens has been shown here to be clearly toxic, and phase II trials of new drugs should pay close attention to the drug safety profile. The aim is to find less toxic treatments and not just treatments that are as toxic but have superior efficacy to standard TB therapy. Adherence, social factors and the effect of other potentially toxic drugs are deciders of the effectiveness of a treatment in the real-world setting (Choi *et al.*, 2014; Kirenga *et al.*, 2014; Tiberi *et al.*, 2018) and unacceptable levels of treatment-associated toxicity will undermine the effectiveness of any therapy.

The majority of adverse events occurred in the first two months of therapy in REMoxTB, and this indicates that shortening the duration of TB therapy will not necessarily translate into less treatment-associated toxicity. The work presented here has clearly demonstrated that the majority of toxic events occurred in the intensive phase of treatment on all three of the treatment arms in REMoxTB, and notably there was no difference between patients receiving placebo (in Months 5 and 6 of treatment) and those patients receiving active treatment on the standard TB therapy arm. Instead, there should be a focus on this early stage of therapy when patients seem to be most vulnerable to treatment-associated toxicity and thought given to the reasons

underpinning this phenomenon. The role of pyrazinamide in this toxicity was raised previously, but the physiological state of the patient at this early stage of treatment should be a consideration (Makhlouf *et al.*, 2008; Hassen Ali *et al.*, 2013; Chen *et al.*, 2015). At the beginning of treatment, it is reasonable to assume that the patient is at their most unwell and this could leave them in a physiologically primed state for developing treatment-related toxicity.

Host-directed therapies and other immune-modulating treatments could be at least part of the solution to this problem (Wallis and Hafner, 2015; Kolloli and Subbian, 2017; Tiberi *et al.*, 2018). There is a renewed interest of late in the effect on treatment outcomes using therapies that up- or down-regulate certain aspects of the host immune system in an attempt to create the optimum immune state for combating active TB disease, as well as the effect of immunosuppressant agents on long term outcomes in pulmonary TB (Madan *et al.*, 2013; Critchley, Orton and Pearson, 2014). Oxidative stress is already known to play a role in drug-related hepatotoxicity associated with TB therapy (W. W. Yew *et al.*, 2018), and therefore it would seem reasonable to hypothesise that the immune state at the beginning of treatment with standard TB therapy would have a bearing on the patient's risk of toxicity. This highlights the need for a broad perspective on how novel drug therapies are developed, with close collaboration between experts in immunology, pharmacology and toxicology.

3.2 Clinical Trial Designs for Novel Tuberculosis Treatments

Safety reporting is a contentious area in clinical trials, in particular the assessment of relatedness for an adverse event, and further work must be done to refine our approach to monitoring adverse events in clinical trials (Events, 2006; Mukherjee *et*

al., 2011; Pocock and Gersh, 2014; Hillman *et al.*, 2017). In this thesis, the proportion of patients experiencing a clinically significant adverse event dropped from 20% to 10%, approximately, when only events assessed as "related" to treatment (i.e. toxicity) were included. This could lead to quite large variation in any estimates of the side effect profile of a new treatment being investigated in a clinical trial. The attribution of relatedness, which relies almost exclusively on the judgement of the treating physician (constrained by soft guidance on the timing of events, effect of any re-introduction etc.), has already been shown as highly variable and often clearly incorrect, such as in situations where a patient is receiving placebo (Hillman *et al.*, 2017).

A more effective approach to the assessment of drug toxicity in clinical trials will have to balance workload for the site doctors, clear attribution of significant toxic events in a reproducible fashion, and regulatory requirements. The majority of adverse events collected in the REMoxTB study were of grade 1 or 2 severity and were considered unrelated to trial medication, yet each one of these thousands of events required the site doctors to complete the relevant paperwork with the same level of documentation as the more severe grade 3 or 4 adverse events. Furthermore, of the events that were considered related to the trial medication the majority were assessed as only "possibly" related.

The result is a collection of recorded events that add little meaningful knowledge to the safety profile of a drug but consume a lot of the site doctor's time and effort. One recommendation following on from the work presented here could be a system whereby only clinically significant events are reported, and relatedness is attributed as either "probably" or "definitely" based on a checklist of agreed criteria. A reproducible relatedness score could be applied (in a similar manner to the RUCAM scoring system (Regev *et al.*, 2014; Danan and Teschke, 2018), but made significantly more brief)

using five to six criteria and the relatedness score deciding the relatedness assessment. The need to convince regulatory authorities on any proposed changes places a burden on the clinical trial community to ensure that any new approach to safety data collection is carefully thought out, involving experienced trial physicians, statisticians and trial managers, and the benefit to participants, site doctors, and the conduct of the trial is made very clear.

4 Final Remarks

The need for new TB treatment options is indisputable: the current standard TB therapy is lengthy, toxic and achieves cure rates below what is needed to end the global epidemic. While there are many drugs in the development pipeline it is unlikely that a novel therapy will be in widespread use any time soon. Therefore, it is critical that we maximise our ability to address the epidemic using the treatment we already have at our disposal. This thesis has sought to contribute to this by adding to our understanding of the toxicity profile of standard TB therapy, especially relating to hepatotoxicity (the most common form of toxicity) and the HIV-positive patient population (a patient group at elevated risk of toxicity).

Close monitoring for liver dysfunction among female patients, those infected with HIV, and those of Asian ethnicity should be considered during the first two months of therapy. By providing a thorough analysis of prospectively gathered data from a large, randomised patient sample receiving standard TB therapy this work can help provide the robust evidence needed to guide individual clinicians and policy-makers. The identification of at-risk patient groups, the most common toxicity, and the time window when toxicity is most likely means that simple plans with clear monitoring schedules can be drawn up and reasonably accurate estimates can be made as to the necessary resources. The demonstration of a significant impact of the rates of treatment failure

will add to the motivation to take seriously the need to address the management of toxic side effects from treatment.

Overcoming the global tuberculosis epidemic will require a multi-faceted approach that involves a combination of better diagnostics, better treatments, and better infrastructure to reach the goals laid out in the End TB strategy. This is an exciting time to be involved in TB research, with more progress made over the past decade than the preceding four decades combined, and it is encouraging to see the global effort to combat this disease. The work presented here will hopefully inform our progress in developing TB treatment by providing detail on the current state of TB therapy, the priorities for future research, and some insight into considerations for the development and trialling on new therapeutics.

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Appendix One: Four-Month Moxifloxacin-Based Regimens for Drug-Sensitive Tuberculosis

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New England Journal of Medicine

2014;371:1577-87.

Appendix Two: Tuberculosis Drug Dosing in the REMoxTB Study, the Roussel-Uclaf Causality Assessment Method, and the Child-Pugh Score

Drug Name		Daily Dosing
Moxifloxacin		400mg
Rifampicin		
	<45 kg	450mg
	>45 kg	600mg
Isoniazid		300mg
Pyrazinamide		
	<40 kg	25mg/kg (rounded to nearest 500mg)
	40 - 55 kg	1000mg
	>55 – 75 kg	1500mg
	>75 kg	2000mg
Ethambutol		
	<40 kg	15mg/kg (rounded to nearest 100mg)
	40 - 55 kg	800mg
	>55 – 75 kg	1200mg
	>75 kg	1600mg

Table 1: Daily dosing of TB medications for patients randomised into REMoxTB based on weight at screening. *Isoniazid and moxifloxacin were both given at fixed doses irrespective of weight*

Criteria		Score
Time to onset of the reaction		
	Highly suggestive	+3
	Suggestive	+2
	Compatible	+1
	Inconclusive	0
Course of the reaction		
	Highly suggestive	+3
	Suggestive	+2
	Compatible	+1
	Against the role of the drug	-2
	Inconclusive or unavailable	0
Risk factor(s) for drug reaction		
	Presence	+1 to +2
	Absence	0
Concomitant drugs		
	Time to onset incompatible	0
	Time to onset compatible, but unknown reaction	-1
	Time to onset compatible and known reaction	-2
	Role proved in this case	-3
	None or no information available	0
Non-drug related causes		
	Ruled out	+2
	Possible or not investigated	+1 to -2
	Probable	-3
Previous information on the drug		
	Reaction unknown	0
	Reaction published but unlabelled	+1
	Reaction labelled in product characteristics	+2
Response to readministration		
	Positive	+3
	Compatible	+1
	Negative	-2
	Not available or uninterpretable	0

Table 2: The Roussel-Uclaf Causality Assessment Method (RUCAM) for causality assessment of adverse drug reactions

Measure	1 point	2 points	3 points
Total bilirubin, µmol/L (mg/dL)	<34 (<2)	34–50 (2–3)	>50 (>3)
Serum albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time, prolongation (s)	<4.0	4.0–6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to severe (or refractory)
Hepatic encephalopathy	None	Grade I–II	Grade III–IV

Table 3: Child-Pugh scoring system for grading the prognosis of chronic liver disease. *Class A: 5-6 points (85% 2 year survival); Class B: 7-9 points (57% 2 year survival); Class C: 10-15 points (35% 2 year survival)*

Appendix Three: Abstracts and Publications Relating to Thesis Output

1 Abstracts

- CD Tweed, G Wills, AM Crook, SK Meredith, AJ Nunn, CM Mendel, SR Murray, TD McHugh, SH Gillespie. Liver function tests during tuberculosis treatment and the implications on monitoring for hepatotoxicity. *Thorax* 2016;**71:**A52-A53
- CD Tweed, G Wills, AM Crook, SK Meredith, AJ Nunn, CM Mendel, SR Murray, TD McHugh, SH Gillespie. Using adverse events in a tuberculosis trial to describe the tolerability of standard therapy. *Thorax* 2016;**71:**A147-A148
- CD Tweed, AM Crook, TD McHugh, CM Mendel, SK Meredith, AJ Nunn, PPJ Phillips, SH Gillespie. Toxicity of TB treatment in HIV-positive patients and treatment outcome: a case control study. *Accepted to 49th Union Conference, The Hague 2018*

2 Publications

- Tweed CD, Wills GH, Crook AM, Dawson R, Diacon AH, Louw CE, McHugh TD, Mendel CM, Meredith SK, Mohapi L, Murphy ME, Murray SR, Murthy SE, Nunn AJ, Phillips PPJ, Singh KP, Spigelman M, Gillespie SH. Liver toxicity

associated with tuberculosis chemotherapy in the REMoxTB study. *BMC Medicine* 2018; 16: 46

- Tweed CD, Crook AM, Amukoye EI, Dawson R, Diacon AH, Hanekom M, McHugh TD, Mendel CM, Meredith SK, Murphy ME, Murthy SE, Nunn AJ, Phillips PPJ, Singh KP, Spigelman M, Wills GH, Gillespie SH. Toxicity associated with tuberculosis chemotherapy in the REMoxTB study. BMC Infect Dis. 2018 Jul 11;18(1):317
- Tweed CD, et al. Treatment Related Toxicity Affecting HIV-infected Patients with Pulmonary Tuberculosis in the REMoxTB Study. *Under review and planned submission to BMC Infect Dis in Nov 2018*